Treatment of phenanthrene and benzene using microbial fuel cells operated continuously for possible in situ and ex situ applications

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Abstract

Bioelectrochemical systems could have potential for bioremediation of contaminants either *in situ* or *ex situ*. The treatment of a mixture of phenanthrene and benzene using two different tubular microbial fuel cells (MFCs) designed for either *in situ* and *ex situ* applications in aqueous systems was investigated over long operational periods (up to 155 days).
For *in situ* deployments, simultaneous removal of the petroleum hydrocarbons (>90% in term of degradation efficiency) and bromate, used as catholyte, (up to 79 %) with concomitant biogenic electricity generation (peak power density up to 6.75 mWm^{-2}) were obtained at a hydraulic retention time (HRT) of 10 days. The tubular MFC could be operated successfully at copiotrophic (100 ppm phenanthrene, 2000 ppm benzene at HRT 30 days) and oligotrophic (phenanthrene and benzene, 50 ppb each, HRT 10 days) substrate conditions suggesting its effectiveness and robustness at extreme substrate concentrations in anoxic environments.
In the MFC designed for *ex situ* deployments, optimum MFC performance was obtained at HRT of 30 h giving COD removal and maximum power output of approximately 77% and 6.75 mWm^{-2} respectively. The MFC exhibited the ability to resist organic shock loadings and could maintain stable MFC performance. Results of this study suggest the potential use of MFC technology for possible *in situ*/*ex situ* hydrocarbon-contaminated groundwater treatment or refinery effluents clean-up, even at extreme contaminant level conditions.

Key words: groundwater, degradation efficiency, microbial fuel cells, petroleum hydrocarbons, copiotrophic.

1. Introduction

Pollution of groundwater by petroleum hydrocarbons is a serious threat to human health as the hydrocarbons are toxic, mutagenic and carcinogenic (UNEP, 2011; Juana et al., 1998). MFCs could be employed in the treatment of these recalcitrant pollutants with concomitant bioelectricity generation (Morris and Jin, 2012). In practical deployments, MFC systems can be deployed in the *in situ* treatment of contaminated groundwater or soils and also as in *ex situ* application, in the treament of industrial effluents such refinery effluents or other effluents from agrochemical industries (Taghavi et al., 2014; Rakoczy et al., 2013). For such practical applications, MFCs would have to be effective, efficient, robust and applied *in situ/ex situ.* MFC systems need to be designed uniquely and tested for their performance before their deployment for either *in-situ* or *ex-situ* deployments.

In wastewater treatment systems, hydraulic retention time (HRT) is an important design parameter which has a significant influence on system design, operational /investment cost, process efficiency and energy requirements (Kuscu and Sponza, 2009; Shariati et al., 2011; Zhang et al., 2012). In general, lower HRTs are desirable from economic viewpoints because higher HRTs will lead to greater investment costs.

There are several studies in which the effect of HRTs on degradation/COD removal efficiency and power density have been studied (Zhang et al., 2012; Jayashree et al., 2014; Akman et al., 2013; Li et al., 2013). Li et al (2013) investigated the effect of HRTs and non-precious metallic catalyst on MFC performance using MFC fed with animal carcass wastewater. They reported a maximum power density and COD removal efficiency of 2.19 Wm^{-3} and 50.66 % respectively

when HRT was set at 3d (days). Most of authors in previous studies had used dual-chambered MFCs which are less efficient (in terms of MFC performance) with longer HRTs compared to tubular MFC design as used in this study. Most of these previous studies used MFC designs that have very limited deployments (i.e. limited to *ex-situ* applications only), also, the cathodic material used are expensive and unsustainable in terms of cost. Tubular MFC designs have recently been reported in the literature as a typical MFC design design that is suitable for practical applications (Taghavi et al., 2014; Kim et a., 2011; Rakoczy et al., 2013).Tubular MFCs could also reduce HRT substantially with respect to dual-chambered MFCs owing to the uniqueness in their designs as previously reported in several studies (Scott et al., 2007; Kim et al., 2011; Taghavi et al., 2014). Kim et al (2011) reported a significant increase in power production to about 5.6 Wm^{-3} with improved organic removal efficiency by increasing the number of modules of extended longitudinal tubular MFC reactors. However, in this current study, a tubular MFC configuration (using bromate as catholyte) was employed to study the effect of HRTs on MFC performance for possible *in-situ* or *ex-situ* applications; studies on the the use of these MFC designs in the treatment of petroleum hydrocarbons using bromate as catholyte have not been reported in the literature to the best of the author's knowledge. This unique MFC design used in the study could help in the reduction of HRT with no significant effect on MFC performance using a cathodic catalyst that is cheap and sustainable.

In the previous studies, the effect of extreme nutrient conditions (such as very low or high nutrient conditions) was not considered in their experimental designs. Nutrient e.g substrate availability is one of the most important factors that directly affects microbial degradation rates/efficiency (Suthersan and Payne, 2005; Haritash and Kaushik, 2009). Good process performance and system stability at these extreme conditions may be an added advantage of MFC technology over conventional anaerobic technologies aside from energy recovery. Therefore, it is necessary to evaluate the effect of substrate availability at extreme levels on MFC performance as reported in this study.

This study investigated the performance of two tubular MFC systems meant for *in-situ*/ *ex-situ* deployments. The MFCs were operated in a continuous mode at different HRTs at ambient temperature. Other important aspects of operation such as toxicity assessment of effluent from the MFCs and the reactor's response to oligotrophic and copiotrophic (substrate) conditions were also investigated.

2. Materials and method

2.1 Chemicals and reagents

Chemicals and reagents were obtained from Sigma Aldrich (Dorset, UK), VWR (UK), Acros (UK) or Fisher Scientific (Loughborough, UK). All chemicals were of analytical grade (≥99 % purity) and used without further purification**.**

2.2 Microorganisms and media compositions

The MFCs were inoculated with petroleum hydrocarbon acclimated mixed microbial population as described in a previous study (Adelaja et al., 2015) from already operating MFCs treating synthetic hydrocarbon-contaminated wastewater. The biomass was centrifuged, washed twice with sterile phosphate buffer before being introduced into the anode chamber at approximately 90 mg wet biomass per anode volume. The hydrocarbon adapted mixed microbial culture was introduced into the MFC units at 10 % of the total reactor working volume. During start-up, the inoculated MFCs were operated in fed-batch mode for three consecutive cycles in order to obtain reproducible MFC performance from all reactors. The same synthetic medium without the petroleum hydrocarbons was used during

the start-up of MFC reactors. *Vibrio fischeri* (13938) used for the toxicity testing was purchased from NCIMB (UK) and was grown in oceanibulbus growth medium (NCIMB growth media catalogue). The defined minimal medium for bioelectrochemical experiments was prepared as follows (per liter of deionized water): 8.24 g Na_2HPO_4, 5.08 g NaH_2PO_4, 1.0 g NH_4Cl, 0.5 g NaCl, 0.25 g $MgSO_4$, 12.5 mL Wolfe trace mineral solution and 12.5 mL Wolfe vitamins solution (Lovely et al., 1984). All media preparations were autoclaved at 121□ for 15 min, except for vitamins, mineral and glucose (2 g L^{-1}) solutions that were filter-sterilized (0.2 µm) (Millipore, UK). The MFCs was fed with a minimal salt media containing 30 ppm phenanthrene (taken from a 1000-fold concentrate in 100% methanol) and 200 ppm benzene as sole substrate during the study of the effect of HRTs on MFC performance. The methanol used in dissolving the phenanthrene (0.01% v/v of the reactor 's working volume) was considered to be non-toxic as previously reported in our earlier studies (Adelaja et al., 2014).

2.3 Reactor designs, configurations and operations.

Two different designs of tubular MFCs were used in this study. The first design (Fig. 1) which would be useful for *in situ* deployment is a tubular two-chamber MFC which was constructed from two concentric polyvinyl chloride (PVC) tubes. The inner concentric PVC tube (inner diameter 4.5 cm x length 40 cm) makes up the cathode chamber with a working volume of 300 mL. The anode chamber (total working volume of 500 mL) comprises of the outer concentric PVC pipe with inner diameter, 6.5 cm and length, 35 cm. Both the anode nd cathode electrodes were constructed from carbon felt (C-TEX 27; surface density 110 g/m^2; surface area 1100 m^2/g, Mast Carbon Inc, Basingstoke, UK) with projected surface area of 156 cm^2 and 96 cm^2 respectively. The anode and cathode chambers were separated by a CMI-7000 cation exchange membrane (Membranes International, USA). Insulated copper wires were used to secure good electrode connections and soldered connection interfaces were carefully insulated with non-conductive, air tight epoxy material. The external circuit of the MFCs was connected across a 1 kΩ resistor to an online data acquisition system (Picolog ADC 24, Pico Technology, UK) and voltage data were logged at an interval of 10 mins. All MFCs (both the tests and controls) were simultaneously and continuously fed using two multi-channel peristaltic pumps (Watson-Marlow, UK) with an up-flow configuration. Potassium bromate, $KBrO_3$ solution (1000 ppm at pH 2) was used as catholyte in the cathode feeding tank whereas the anode feeding tank was filled with petroleum hydrocarbon containing synthetic wastewater. The petroleum hydrocarbon containing wastewater and bromate solution influent feeding rates were varied simultaneously throughout the study (section 2.4). All tubular MFCs were operated continuously at ambient temperature which varied from 14□ and 23□ corresponding to winter and summer periods respectively.

Notably, all the tubular MFCs (i.e. both MFC designed for *in situ* and *ex situ* deployments) were seeded (at 10 % of the total reactor working volume) with inoculum taken from a running MFC (designated for sole purpose). The biomass was centrifuged, washed twice with sterile phosphate buffer before being introduced into the anode chamber at approximately 0.6 OD (optical density) at 600 nm per anode volume (500 mL). The running MFC was previously seeded with an inoculum of the adapted mixed culture previously described in section 2.2. During start-up, the inoculated MFCs were operated in fed-batch mode up-to three consecutive cycles in order to obtain reproducible MFC performance from all replicate reactors.

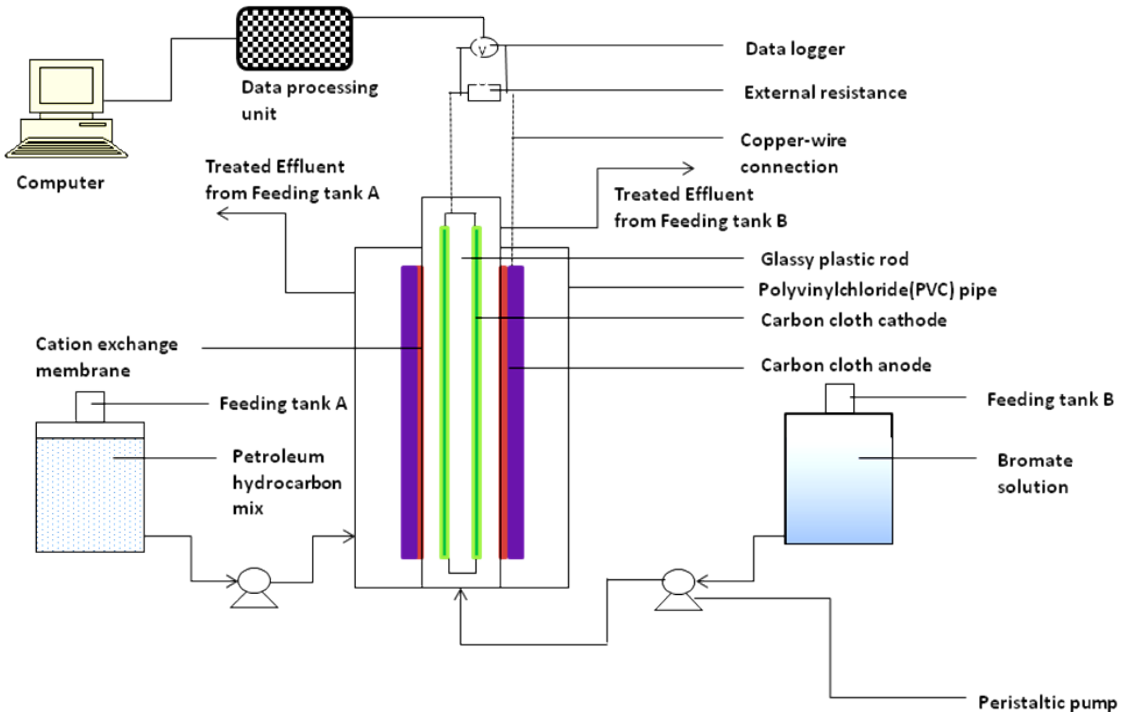


Fig. 1: A schematic diagram of the tubular MFC reactor in an up-flow configuration for the treatment of hydrocarbon-containing synthetic wastewater for *in situ* applications.

The second MFC designed for possible *ex situ* deployment was a tubular single chamber MFCs constructed from PVC pipes and had a total working volume of 400 mL (Fig. 2). The MFC reactor dimensions were 3.5 cm (inner diameter) and 60 cm length. The anode and the cathode were made of carbon felt material (C-TEX 27; surface density 110 g/m^2; surface area 1100 m^2/g, Mast Carbon Inc, Basingstoke, UK) with surface areas of 192 cm^2 (projected) and 128 cm^2 (measured) respectively. The CMI-7000 cation exchange membrane (Membranes International, USA) was emplaced between the anode and the cathode and electrode spacing between them was approximately 1.3 cm.

The hollow concentric anode ran through the length of the PVC tubes for both reactor designs. Pt powder was used as the oxygen reduction catalyst on the cathode and was coated onto the cathode membrane-facing side at Pt/C loading of 0.35 mg/cm^2. A Pt/C mix was prepared by carefully mixing Pt powder with carbon black powder (Sigma Aldrich, UK) in a ratio of 1:10 (i.e. 10 % Pt/C w/w). Subsequently, the 10 % Pt/C mixture was suspended in Nafion binder solution (Sigma Aldrich, UK) and its suspension was later applied as a uniform coating on the cathode electrodes using a paint brush. The air facing side of the cathode contained a PTFE diffusion layer in order to minimise water loss through the cation exchange membrane. The PTFE gas diffusion layer was applied as described by Antolini et al (2002).

The connections on the electrodes were secured using insulated copper wire soldered to the electrodes. After insulation of the soldered electrodes, the resistance between the electrode and wire was verified and ensured to be less than 20 Ω in order to minimise ohmic losses due to high internal resistance of the MFCs. The MFCs were connected to a 1 kΩ external resistor and the voltage across the resistance was recorded every 10 mins using the data logging system. Petroleum hydrocarbon- wastewater was fed continuously (plug-flow velocity profile) to the MFCs using a multi-channel peristaltic pump (Watson-Marlow, UK). The hydrocarbon containing influent feeding rate was varied throughout the study described in Section 2.4.

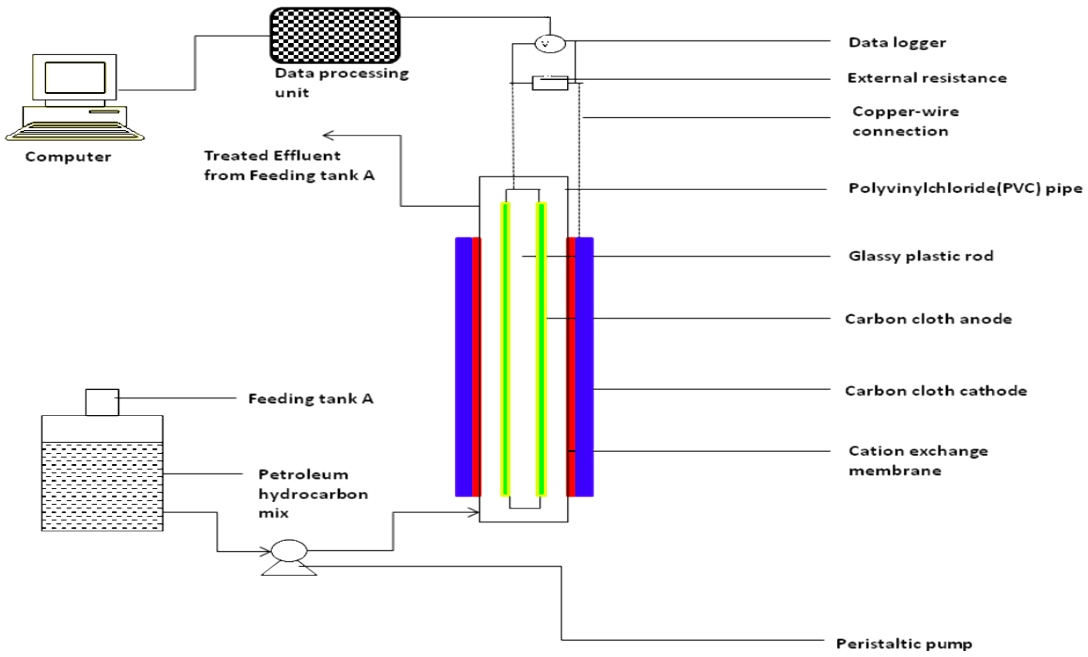


Fig. 2: Schematic diagram of the tubular MFC reactor in an up-flow configuration used for the treatment of petroleum hydrocarbon containing synthetic wastewater for a possible *ex situ* application.

2.4 Experimental design

2.4.1 Eperiment 1 involving tubular MFCs designed for *in-situ* applications

The experimental set-up consisted of four identical tubular MFC units (Fig. 1) operating in continuous flow mode, two of which were duplicate tests while the other two was an open-circuit control and a non-MFC (or anaerobic) control. These MFC units were operated as previously described in section 2.3. HRTs were decreased stepwise from 10, 5, 2.5 to 2 days only after at least a throughput of three reactor volumes had been achieved. The stability and subsequent recovery of MFC systems during extreme nutrient (substrate) conditions were also investigated. Two extreme conditions considered were oligotrophic (phenanthrene and benzene, 50 ppb for each) conditions and copiotrophic (1500 ppm benzene and 100 ppm phenanthrene) conditions. MFCs were operated continuously under those extreme nutrient conditions at a constant HRT of 10 days. Samples were drawn from all reactors and feeding tanks at set time points (every three days interval) throughout about 155 days of MFC operation and reactor performance was monitored in terms of petroleum hydrocarbon and COD removal efficiencies.

2.4.2 Experiment 2 involving tubular MFCs designed for *ex-situ* applications

In another study, a different MFC design, the tubular single chamber MFC (Fig. 2) was employed in the treatment of petroleum hydrocarbon-containing wastewater at different HRTs in a continuous mode operation. The design and operation of this single chamber MFC reactor have been described in section 2.3. Three MFC units were set-up with two of them as tests (i.e. in duplicates) while the last one was an open-circuit control. The HRTs were incrementally varied from 10 h to 30 h at ambient temperatures ranging between 15□ and 25□ during MFC operations. HRTs of the MFCs were changed after achieving at least a throughput of three reactor volumes. The influent in the feeding tank was made of minimal medium supplemented

with 30 ppm phenanthrene and 200 ppm benzene. The potential for MFC recovery (in terms of reactor performance) and ability to withstand sudden shock-loads of petroleum hydrocarbons were investigated. MFCs was fed with the mixture (100 mg L^{-1} phenanthrene and 1500 mg L^{-1} benzene in minimal medium and were operated continuously at a constant HRT of 30 h. Ten (10) mL of the samples were drawn from all reactors and feeding tank at 12 hrs intervals throughout MFC operations. Reactor performance was monitored in terms of power outputs, petroleum hydrocarbon and COD removal efficiencies

2.5 Analytical methods

2.5.1 Chemical analysis

2.5.1.1 Petroleum hydrocarbon determination

Anolyte samples containing petroleum hydrocarbons (phenanthrene and benzene) were analyzed by high-performance liquid chromatography (HPLC, Dionex GS50, USA) using a Photo-diode Array (PDA) detector (DIONEX, PDA-100) at 254 nm. The injected volume was 20 µL with column oven temperature (25□) and the HPLC was operated at isocratic conditions. The analytical column was a reverse 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^{-1}. The minimum detectable concentration for benzene and phenanthrene was 50 µg L^{-1} and 5 µg L^{-1} respectively.

Extractions of petroleum hydrocarbons present in the anolyte samples and on the electrode were carried out as described by Kermanshahi pour et al (2005). Approximately 1 mL of aliquots were withdrawn at intervals from the MFCs (including the aqueous phase of the soil MFCs) and transferred to a 2 mL eppendorf tube. Subsequently, 1 mL of methanol was added to make 2 mL and these were incubated on a shaker for 1 h at 25□ and 150 rpm. Eppendorf tubes were immediately centrifuged at 13.2 x g for 10 mins and 1 mL of supernatant was carefully transferred into 1.5 mL HPLC glass vials prior to analysis by HPLC. The benzene concentrations in the gaseous phase were calculated with Henry's law using the constant at 25□ of 0.25 for benzene (Zhang et al., 2010). Degradation efficiencies and rates were determined based on the remaining petroleum hydrocarbons in solution at different time intervals during the continous MFC operation.

2.5.1.2 Determination of bromate removal in MFCs

The amount of bromate removal was determined quantitatively by using a spectrophotometric method as described by Emeje et al (2010). One mL of freshly prepared 0.5 % potassium iodide (KI) solution in 0.1 M hydrochloric acid (HCl) was added to I mL of the sample; the mixture was then vortexed for 2 mins and allowed to stand for 10 mins at room temperature (25 ± 5□). The presence of potassium bromate was indicated by change in colour from light yellow to purple/pale brown. The absorbance of the sample was taken at 620 nm in a UV-Vis spectrophotometer M 6300 model (Jenway Staffordshire, UK).

Bromate solution with given concentrations (from 0 to 1000 ppm) were prepared by the dilution of potassium bromate ($KBrO_3$) solution (Sigma Aldrich, UK) with deionised water for calibration curve generation. Calibration standard curve of pure bromate was used to quantify the concentration of the samples using the absorbance readings. The concentration of bromate consumed was expressed as percentage bromate removal.
The percentage bromate removal was calculated as follows;

Percentage bromate removal (%) = $\frac{Br_i - Br_f}{Br_i} \times 100$..... (1)

where Br_i and Br_f are initial bromate and final bromate concentrations respectively.

2.5.3 COD removal

The chemical oxygen demand (COD) of the samples was determined using the closed reflux titrimetric method as described in the Environment Agency (UK) Standard method 5220 D (APHA, 1997). Briefly, the samples were centrifuged at 6000 *g* for 10 mins at 5□ and the supernatant was filtered through a 0.22 μm PTFE filter in order to remove suspended biomass. Appropriately diluted 1 mL samples were added to 4 mL of Ficodox mixed COD reagent, vortexed for 2 mins and digested on a pre-heated heating block for 2 h at 150°C in closed digestion tubes. A reagent blank containing 1 ml of deionized water treated with the same reagent as the sample was digested with each set of samples. After 2 h, the digested samples were cooled to room temperature (25 ± 2□). Subsequently the digestate was transferred to a conical flask and 2-3 drops of Ferroin indicator solution (Fisher Scientific, UK) was added. A 0.025M ferrous ammonium sulphate (FAS) titrant was used with the tritrand (digestate samples) in order to titrimetrically determine the residual volume of the Potassium dichromate contained in the Ficodox digestate.
COD removal was calculated as;

$COD\ (mg\ L^{-1}) = (K_b - K_s) \times DF \times M \times 8000$……..…. (2)

where, K_b and K_s are ferrous ammonium sulphate (FAS) titrant volumes for blank and the sample respectively, DF is the sample dilution factor and M is the molarity of the FAS solution. The COD of samples was expressed as percentage COD removal. The percentage COD removal was calculated as follows:

Percentage COD removal (%) = $\frac{COD_i - COD_f}{COD_i} \times 100$ .....(3)

where COD_i and COD_f are initial COD and final COD values respectively for each experiment.

2.5.4 Electrochemical analysis

The performance of the MFCs for all studies was assessed based on voltage and current outputs. Electric current (I) flowing through the external load was estimated using the employed resistance (Ω) and measured potentials (E). Polarisation curves were obtained by changing the external resistances from 1 Ω to 1 MΩ across the external circuit at average interval of about 5 mins after the MFC reached a stable cell potential. The current flowing through each external load of the MFC and power produced were determined as described by Logan (2008). The total internal resistance (R_{int}) of the MFC systems were estimated using the polarisation slope method as described by Logan et al (2006) and Fan et al (2008). The maximum power density was obtained using the power density curve method (Logan, 2008).
Power density *P* (mWm^{-2}) was calculated as;

$P = \frac{I \times E}{A}$ ……..…(4)

where I (mA) is the current, E (mV) is the voltage and A (m^2) is the projected surface of the anode. Power density (Wm^{-2}) and current density (Am^{-2}) were normalized to the projected total surface area of the anode.

Coulombic efficiency (CE) was calculated as

$$CE\ (\%) = \frac{\int I\,dt}{C_t} \times 100 \quad \text{..... (5)}$$

where $\int I\,dt$ is the coulombs calculated by integrating the current over time (t), C_t (C) is the theoretical amount of coulombs that is available from COD, which was calculated as $C_t = FbV\Delta COD/M$, where F is the Faraday's constant (96485 $Cmol^{-1}$), b is the number of moles of electrons produced per mol of substrate (b = 4), V is the working volume of the anode (L), ΔCOD (mg COD L^{-1}) is the change in COD concentration, and M is the molecular weight of the substrate (M= 32) (Sleutels et al., 2011).

2.5.5 Bioluminescence toxicity assays

Toxicity assays were performed according to the Microtox standard acute toxicity testing procedure (Gaudet, 1994). A bioluminescent marine organism, *V.fischeri* was grown in Oceanibulbus medium for 72 h in an incubator set to 22□ and 150 rpm before the cells were harvested by centrifugation at 6000 *g* for 15 mins. The cell pellet was washed twice with sterile phosphate buffer (50 mM, pH 7) and was re-suspended in a sterile 2 % NaCl solution before use in the toxicity assay. All samples analysed were centrifuged at 13.2 x *g*, filtered through 0.22 µm PTFE filters to remove suspended biomass. Exactly 2 % NaCl was added to all samples prior to the test procedure for osmotic adjustment of samples. The luminescent intensity measurements of samples were taken using Fluostar Optima (BMG Labtech, Ortenburg, Germany) luminometer. The sample incubation temperature was set to 25□ and samples incubated for 15 mins prior measurement. The half-maximal effective concentration, EC_{50} (indicating the concentration at which a 50 % reduction in luminescent intensity was observed compared to controls) was expressed as a COD equivalent of the analysed samples.

2.6 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 standard deviation of the mean (SD).

3. Results and discussion

3.1 Treatment of petroleum hydrocarbons using a novel two chambered-tubular MFCs

3.1.1 Effect of different HRTs on power generation and degradation performance of the novel tubular MFC during continuous operation.

The changes in degradation performance and bioelectricity generation were monitored over the course of the reactor operation with gradual decrease in HRT from 10 - 2 d (Fig. 3 and 4). COD reduction was observed (effluent concentration even as low as 200 mg L^{-1}) at different HRTs over the period of continuous MFC operation. Notably, COD reduction in the effluent of the reactor was significantly lower than that of the open circuit control (analogous to a conventional anaerobic process).

As indicated in Fig. 3B, removal efficiencies higher than 90 % for both phenanthrene and benzene were observed. The stepwise decrease in HRT during continuous reactor operation did not adversely affect petroleum hydrocarbons removal efficiency of the reactor system and high (> 90%) removal efficiencies were attained even at the highest loading rate (700 mgCOD/L/day; HRT of 2 d). The reactor system was capable of sustaining the high removal rates irrespective of the HRT or pollutant loading rates throughout the long-term operation (more than 100 days); hence indicating the robustness of the reactor system.

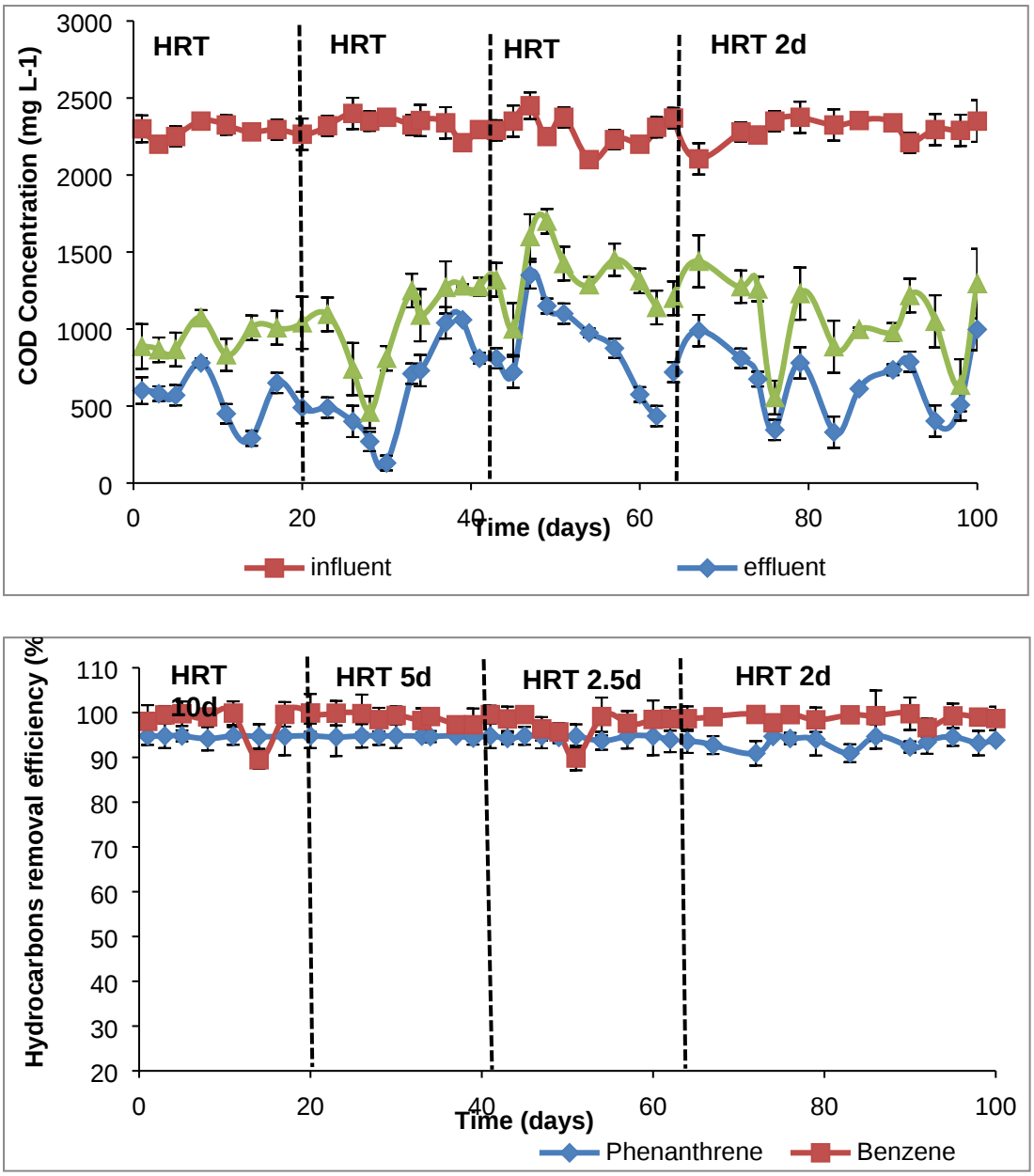


Fig. 3: (A) COD removal and (B) Hydrocarbons removal efficiencies at various HRT regimes over a period of 110 days of continuous MFC operation at ambient temperatures (14-25 □) using adapted microbial consortia. Values are means of duplicate experiments ± SD.

These observations are consistent with results reported previously for petroleum hydrocarbons or organic pollutant removal at different HRTs in MFC systems (Zhang et al., 2012d; Li et al., 2013; Feng et al., 2014; Akman et al., 2013; Kuscu and Sponza, 2009). The MFC system was operated at ambient temperature and temperature fluctuations had little or no apparent effect on petroleum hydrocarbon removal efficiency throughout the period of continuous MFC operation, even during winter period.

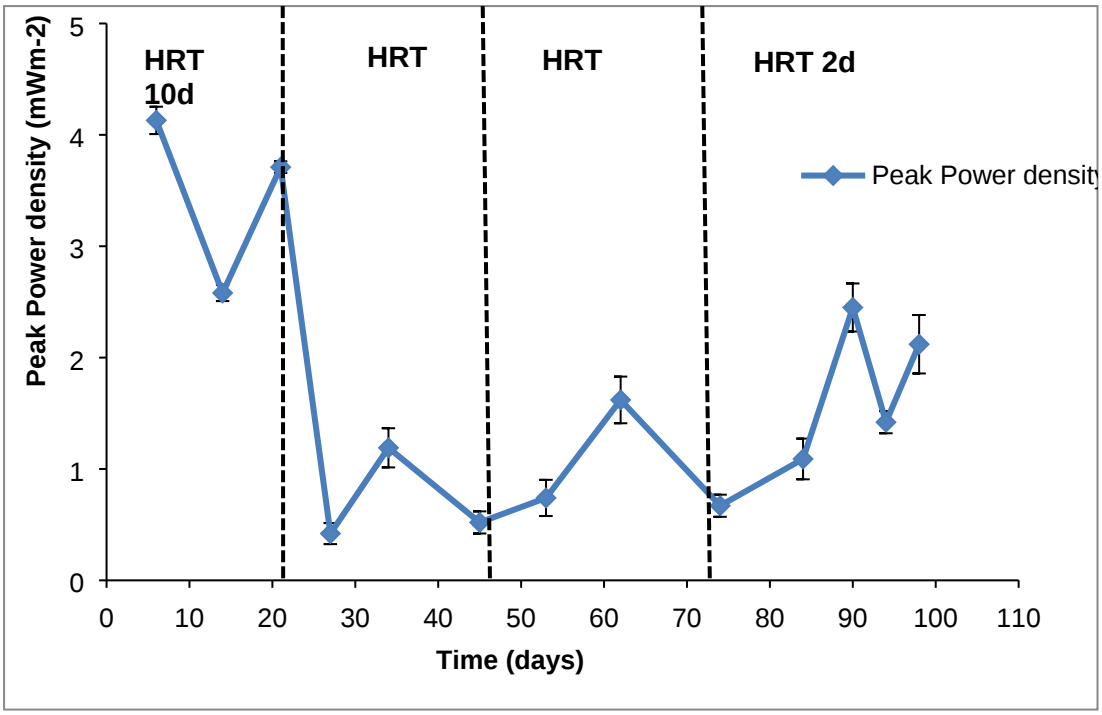


Fig. 4: Profile of peak power densities produced at different HRTs in a tubular MFC over 100 days continuous operation at ambient temperatures (10-25 □) using adapted microbial consortia. Values are means of duplicate experiments ± SD.

There was sharp decrease in average power outputs as HRT was changed from 10 days to 5 days and thereafter a steady rise in power production was observed with stepwise decrease in HRT (Fig. 4). With regards to COD removal, average total COD removal efficiencies decreased significantly from 74 % to 57 % with decreasing HRT. The minimum average total COD removal efficiency (57 %) was observed at HRT 2.5 days as shown in Fig. 5. Open circuit control indicated an open circuit voltage (OCV) of 549 ± 27 mV throughout the study (data not shown). However, there was an overall decrease in power densities with decreasing HRT over the long period of operation. The observed trend in power outputs, particularly at HRT of 5 d with lowest power density of 1.06 mWm^{-2} may be attributed to change in ambient temperature.

The period that the HRT of the MFC system was changed from 10 to 5 d fell within the winter seasons where very low temperature of 10 -16□ were recorded. This low ambient temperature may cause a shocking effect on the microbial physiology or biokinetics thus leading to decrease in power generation (Larrosa-Guerrero et al., 2010; Fernando et al., 2013). Notably this observation was further corroborated by corresponding decrease in COD removal temporarily. As the ambient temperature increases, due to change of seasons and microbial adaptation, a steady recovery in power outputs was observed which corresponded with a slight increase in COD removal efficiency(Li et al., 2013; Fernando et al., 2014).

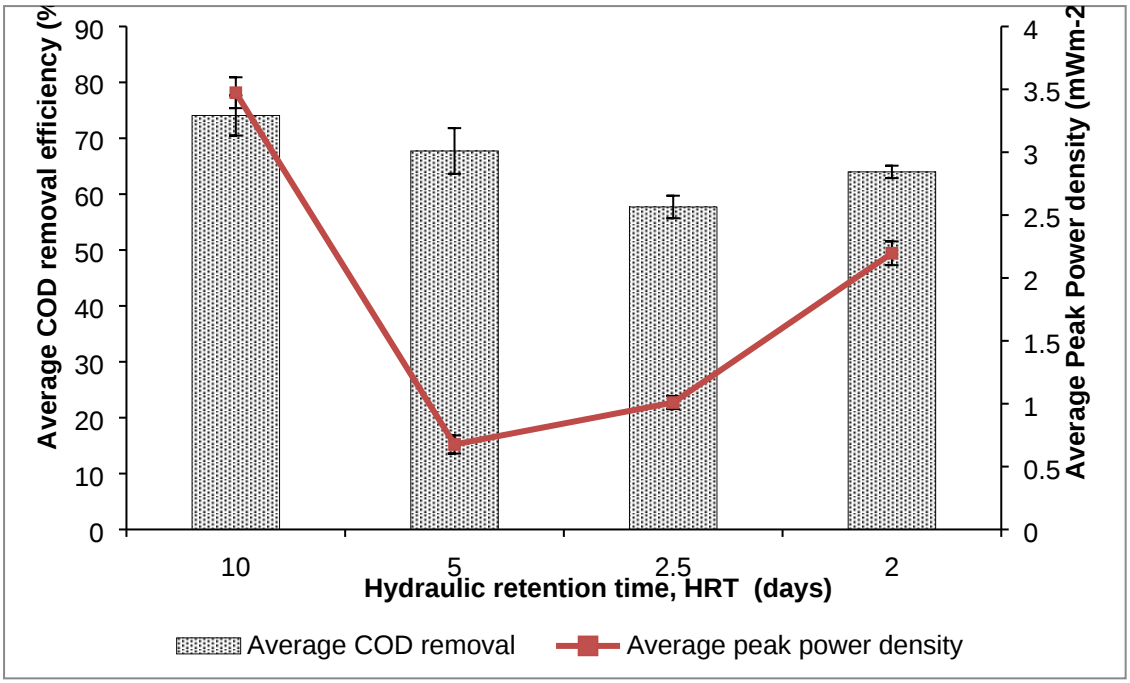


Fig. 5: COD % removal and peak power density over the test period in MFCs at different HRTs in the novel tubular MFC at ambient temperatures (10-25□) using adapted microbial consortia. Error bars ± SD are based on duplicate measurements.

These observations corroborated well with the results of numerous other studies that reported increase in COD removal efficiencies at higher HRT regimes (Kuscu and Sponza, 2009; Akman et al., 2013; Li et al., 2013) and this can be attributed to the decrease in organic loading rate (OLR) with increasing HRT. This suggests the potential practical deployments of this tubular MFC reactor system for degradation of petroleum hydrocarbons coupled with concomitant bioelectricity production in sub-surface environments where conventional technologies such as permeable reactive barrier (PRB) and *in situ* chemical oxidation (ISCO) technologies are proven ineffective and unsustainable (in terms of remediation costs and strategy).

3.1.2 Simultaneous bromate removal during MFC operation.

In the cathode chamber of the MFC reactor, bromate was used as a terminal electron acceptor in lieu of a platinised cathode. From Fig. 6, it can be seen that bromate removal efficiency rose from 52.5-78.6 % as HRT was raised from 2-10 d indicating that most of the bromate ions (BrO_3^-) removed was electrochemically reduced to bromide ions (Br^-). A possible explanation for the marked increase in bromate removal could due to the increase in residence time which allowed more electrochemical transfer of electrons (resulting from the anodic oxidation in the anode) to be used in the reduction of bromate ions to bromide ions. Bromate ion is a well-known toxic pollutant which is classified as carcinogenic in nature (especially to humans) by the International Agency for Research on Cancer (IARC) and the WHO standard for drinking water is 0.01 mg L^{-1} (Zhao et al., 2012). The conversion of bromate ions through bioelectrochemical processes (as used in this study) to non-toxic and bromide ions which is naturally present in most water bodies is a promising method of bromate removal from the environment in a cost effective and sustainable manner as achieved in this study. This is the very first study that has demonstrated simultaneous treatment of two pollutants in both

chambers (i.e. petroleum hydrocarbon and bromate removal at the anode and cathode respectively).

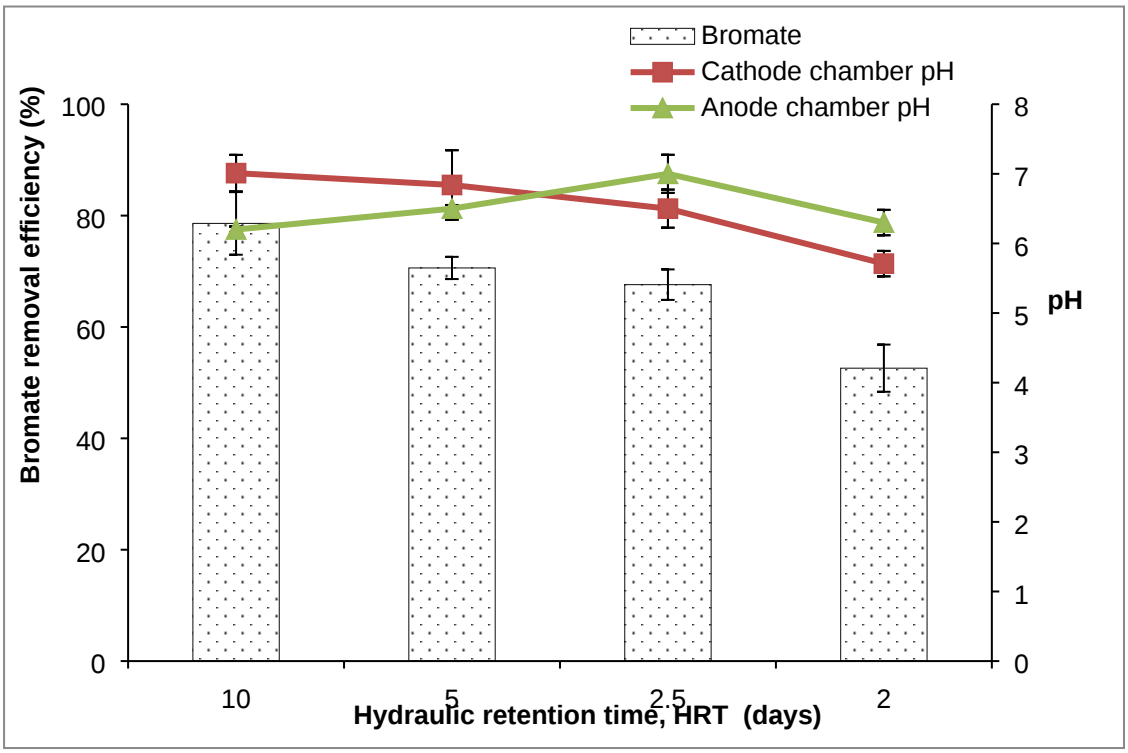


Fig. 6: Bromate removal performances and changes in pH in the novel tubular MFC reactor over 110 days operation at ambient temperatures (10-25□) using adapted microbial consortia. Error bars ± SD are based on duplicate measurements.

The standard potential of BrO_3^- and HOBr is given as follows (Zhao et al., 2012):

$BrO_3^- + 5H^+ + 4e^- \rightleftharpoons HOBr + 2H_2O$ 1.447 V vs SHE ..... (6)

$HOBr + H^+ + 2e^- \rightleftharpoons Br^- + H_20$ 1.341 V vs SHE .....(7)

The initial pH of catholyte (bromate) was 4.55 which is moderately acidic due to the formation of bromic (I) acid (HOBr) in solution especially in the presence of protons migrating from the anode. Bromic (I) acid (HOBr) may be more reactive than BrO_3^- because anions are generally difficult to approach the cathode because of electrostatic repulsive forces (Zhao et al., 2012). During continuous MFC operation, bromate ions in the cathode chamber are reduced to bromide ions which can be either adsorbed on the cathode (a carbon electrode) or evolved as bromine gas due to the reaction between two bromide ions which is dependent on the redox potential and electrocatalytic activity occurring in the cathode chamber (Zhao et al., 2012; Von Gunten et al., 1996). During this process, the pH of the catholyte was raised which also depends on the concentration of bromate ions or bromic (I) acid present in the catholyte. The observed downward trend in pH of the cathode is consistent with decrease in bromate removal performance. Slight increase in anode pH which indicates the formation of secondary/intermediate metabolites resulting from microbial anaerobic oxidation process occurring in the anode chamber is negatively correlated with the decrease in bromate removal efficiency and cathodic pH as the HRT was changed from 10 to 2 d.

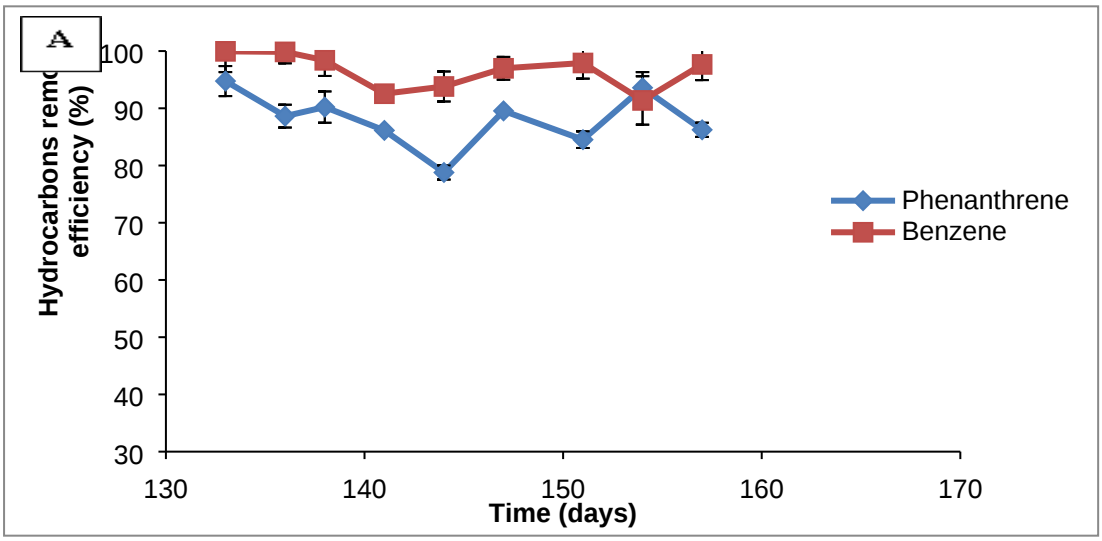
Considering that the high cost of platinum and possible catalyst poisoning or wash off (especially in environmental matrices) have profoundly limited its use in large scale MFC operations (You et al., 2006; Rabaey et al., 2004), bromate contaminated water could be a potential replacement for Pt cathodes in MFCs as previously demonstrated in earlier studies (data not shown). However, this current study has demonstrated simultaneous removal of bromate and petroleum hydrocarbons with energy recovery in the tubular MFC reactor. Findings of this study suggest the potential application of this tubular MFC prototype design for treatment of contaminated groundwater especially in deep aquifers where the deployment of MFC platinised cathode will be ineffective due to the lack of air or oxygen.

3.1.3 Effect of oligotrophic and copiotrophic conditions on MFC operation

For a continuous bioreactor to be deployed for potential *in situ* deployment especially in contaminated groundwater treatments, it is necessary for the bioreactor to possess the ability to withstand extreme or harsh nutrient or substrate loading conditions. Particularly, in groundwater environments, it is very likely to encounter wide variation in organic loading rates ranging from very low to exremely high pollutant and COD levels. Therefore, any potential MFC-based system developed for this purpose must be robust enough to withstand such harsh conditions. Hence, as part of this study, an experiment was set-up to investigate the performance of a tubular MFC operated in a continuous mode (with HRT of 10 days) at oligotrophic and copiotrophic (substrate) conditions under ambient temperature (14-22□).

Good MFC performance (in terms of COD removal effciency) was obtained between 65 and 95 % at both extreme nutrient conditions (Fig. 7). Maximum power density of 0.76 mWm^{-2} was obtained under copiotrophic condition. Furthermore, degradation efficiencies of phenanthrene and benzene were maintained above 80 % and 90 % respectively even at high pollutant levels. However, power outputs were very low (0.01 mWm^{-2}) especially under oligotrophic condition. The concentration of petroleum hydrocarbons after treatment were below the instrument's dectection limit therefore, data on their degradation efficencies at such low substrate levels were not available.

Most of the previous studies reported in the literature have focused on effect of high organic loading or shock load on bioreactors (including MFCs) at different operating conditions (Fernando et al., 2014; Senturk et al., 2012; Karim and Gupta, 2006; Jayashree et al., 2014). However, at very low COD/pollutant levels, substrate concentration can become a rate limiting factor to microbial degradation.



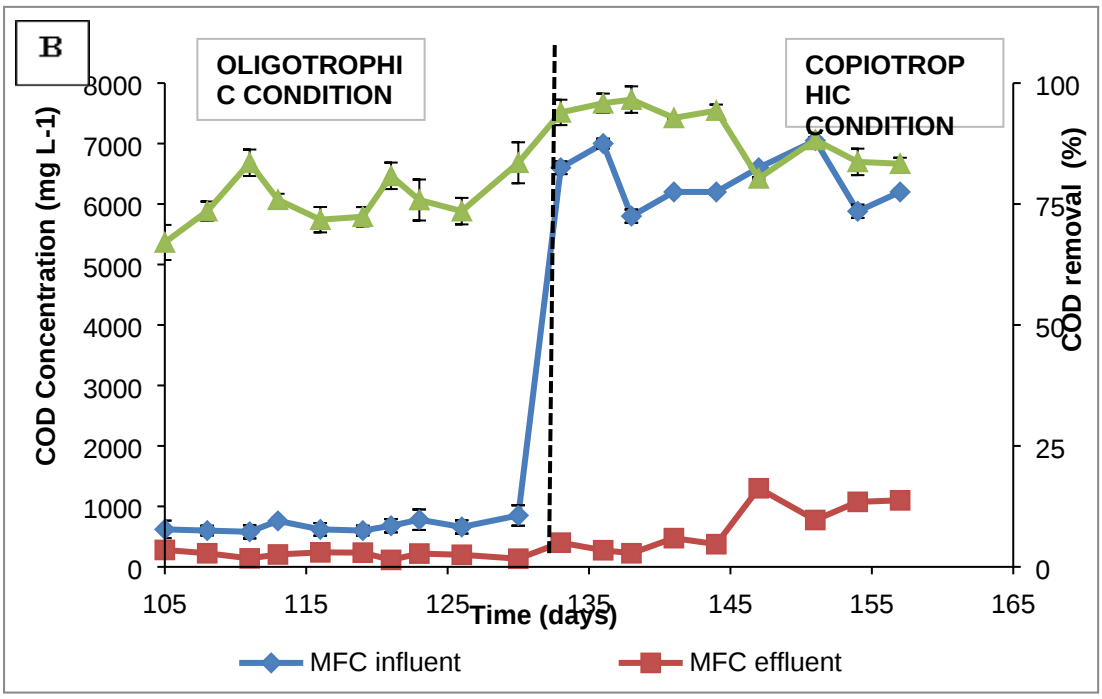


Fig. 7: (A) Degradation performance of the tubular MFC at copiotrophic (substrate) conditions (B) COD removal at oligotrophic and copiotrophic (substrate) conditions operated in a continuous mode at HRT of 10 days. Error bars ± SD are based on duplicate measurements.

In a continous bioreactor, biomass/cells wash out might occur over an extended period of time time (probably after one to two column throughputs) in very low substrate (oligotrophic) conditions. However, since MFC reactors are biofilm-based technologies, microorganisms present in the anode biofilm might be able to effectively utilise the substrate. At this low substrate conditions, substrate adsorption at the electrode could also help to facilate the localisation of substrate molecules thus increasing substrate concentraton at the anode.

In this MFC reactor, at oligotrophic condition, power production was extremely low indicating very little or dormant microbial acitivity taking place. It is likely possible that the biofilms formed on the anode adapted itself to this harsh condition by allowing the microbial cells at the outer surface to switch off or become dormant (in order to preserve itself) while protecting the microbial colony present within the inner part of the biofilm (Juana et al., 1998; Fernando et al., 2014; Akman et al., 2013). This speculation was further supported by fast recovery in overall MFC performance as the MFC was switched to copiotrophic conditions.

These results imply that the tubular MFC was capable of withstanding both shock loadings (high concentration) and very low substrate concentrations even at ambient temperatures. Therefore, these findings suggest that the MFC could be deployed for potential *in situ* deployment in contaminated groundwater treatments due to its effectiveness and robustness even at extreme conditions as demonstrated in this study.

3.1.4 Toxicity reduction during MFC operation

The ultimate goal of any remediation process depends strongly upon achieving a low environmental toxicity of the effluents of treated wastewater which should be either below or within the acceptable limits set by relevant regulatory agencies (Ayed et al., 2011; Andrew, 2010). In most instances where microbial degradation of recalcitrant compounds occurs,

intermediate/metabolites products are formed which may even be more toxic than the parent pollutant. Results of this study indicated that the bioluminescence reduction toxicity effect on *V.fischeri* cells decreased with increase in HRT from 2 to 10 days. The MFC effluents at both HRTs were generally less toxic than the influent as shown in Fig. 8.

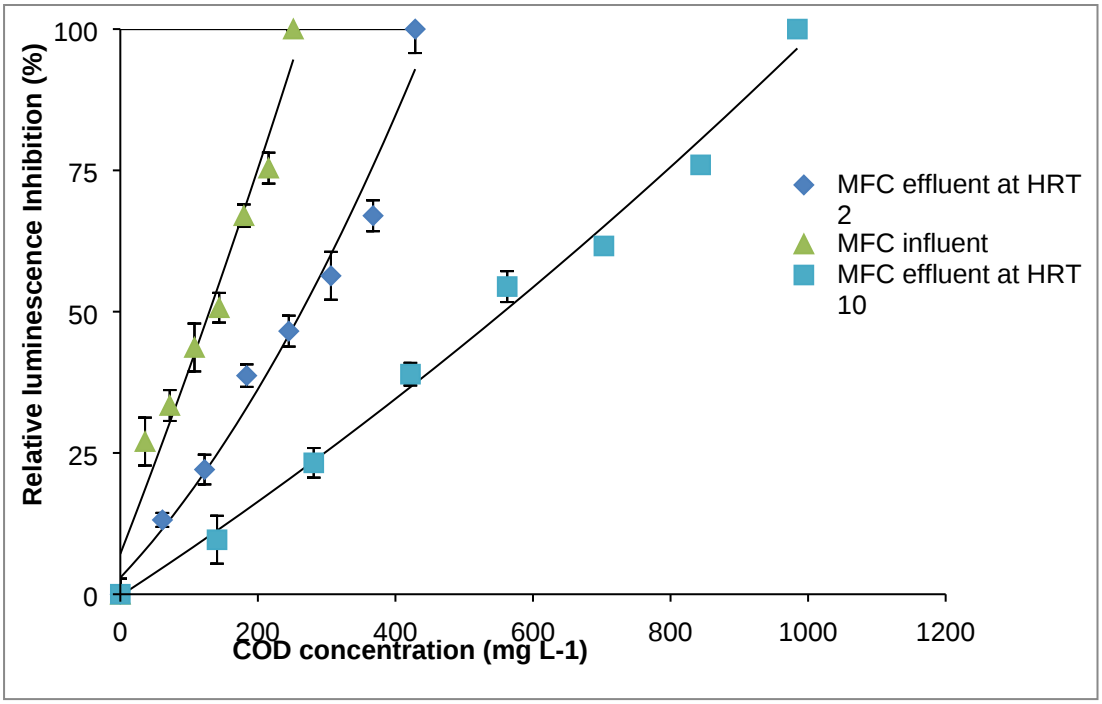


Fig. 8: Bioluminescence based toxicity determinations of MFC effluent and influent at HRTs 10 and 2 days using bioluminescent marine bacteria, *V.fischeri*. Values are means of duplicate experiments ± SD.

The half maximal luminescence inhibition value (EC_{50}) for the petroleum hydrocarbon containing synthetic wastewater influent and MFC effluents at HRT 2 and 10 d were 110 mgCOD L^{-1}, 226 mgCOD L^{-1} and 587 mgCOD L^{-1} respectively. Notably, the MFC effluent at HRT 10 d indicated a marked increase in the EC_{50} value (587 mgCOD L^{-1}) suggesting a significant ($p < 0.01$, ANOVA) reduction in toxicity compared to both MFC influent and MFC effluent at HRT 2d. A possible explanation for this significant decrease in toxicity of the MFC effluent at HRT 10 d which was approximately 4-fold lower compared to the influent could be attributed to longer residence time between the microbes and the substrate within the reactor thus leading to further breakdown of the metabolic products into less harmful products with very low cytotoxicity effects (Li et al., 2013; Feng et al., 2014).

Interestingly, the low EC_{50} value (high toxicity) of the petroleum hydrocarbon contaminated wastewater influent suggests that the presence of unreduced petroleum hydrocarbons (in this case, the presence of unreduced form of phenanthrene and benzene) in high concentrations may contribute to high environmental toxicity and can be carcinogenic in nature due to high degree of aromaticity (Cho et al., 2004; Juana et al., 1998).

Therefore, the results suggest that environmental toxicity of the samples were considerably reduced by MFC treatment especially at relatively high HRT (i.e. 10 d). These findings corroborate well with few other previous studies where a reduction of toxicity was observed following anaerobic treatment of petroleum hydrocarbon contaminated wastewater (Xiong et al., 2012; Cooper et al., 2010; Bautista et al., 2009; Nipper et al., 2005). Toxicity data together

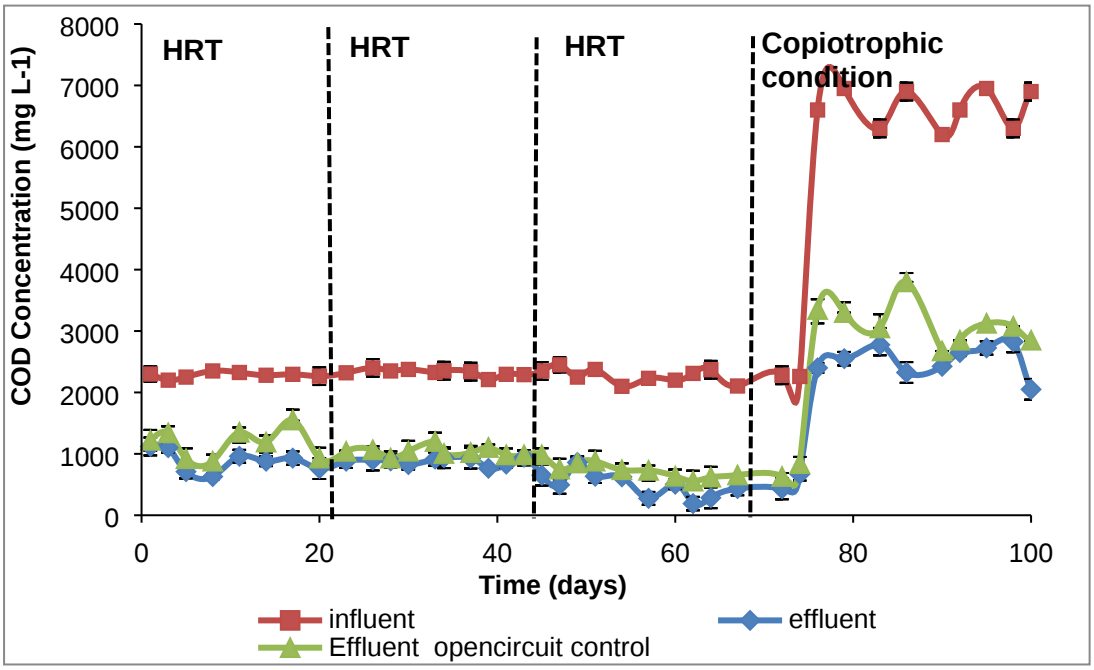
with other experimental evidence provided earlier in sections 3.1.1 and 3.1.3 further demonstrate the effectiveness of this novel tubular MFC in the *in situ* treatment of petroleum hydrocarbons especially in deep aquifers with contaminated groundwater.

3.2 Experiment 2: Effect of HRT on MFC performance using a single chamber-tubular MFC fed with petroleum hydrocarbon-containing wastewater for *ex situ* applications.

3.2.1 Performance under copiotrophic (substrate) conditions.

The tubular MFC was operated at different HRTs (10 -30 h) and the effect of shock loading was investigated in order to evaluate the degradation performance of the MFC system under these conditions. The COD reduction in MFCs over different range of HRTs during the period of continuous MFC operation is shown in Fig. 9A. A consistent drop in COD levels (from 100 - 20 mg L^{-1}) was observed as HRT was changed from 10 to 30 h. which was significantly ($p < 0.05$) higher compared to the MFC open circuit control over the incubation period. Average COD removal efficiencies and peak power densities increased progresssively from 60 to 77 % and 4.72 to 6.75 mWm^{-2} respectively when HRT was raised from 10 to 30 h (Fig. 10). Meanwhile, petroluem hydrocarbon removal efficiences consistently stood above 90 % over the range of HRTs investigated, indicating that the degradation efficiency was not significantly influenced by change in HRT regimes. Optimum MFC performance was obtained at HRT of 30 h giving COD removal and maximum power output of approximately 77 % and 6.75 mWm^{-2} respectively.

HRT is an important parameter in wastewater treatment, determining the content of effluent substrate and power generation in the MFC. As previously discussed in section 3.1.1, high HRT infers more contact time between the microbes and the substrate within the MFC reactor which may have reflected in higher COD removal efficiency observed at higher HRT of 30 h compared to 10 h. Similar observations have been reported by numerous authors in previous studies on the effect of HRTs on MFC performance (Gong and Qin, 2012; Zhang et al., 2012d; Li et al., 2013; Feng et al., 2014).



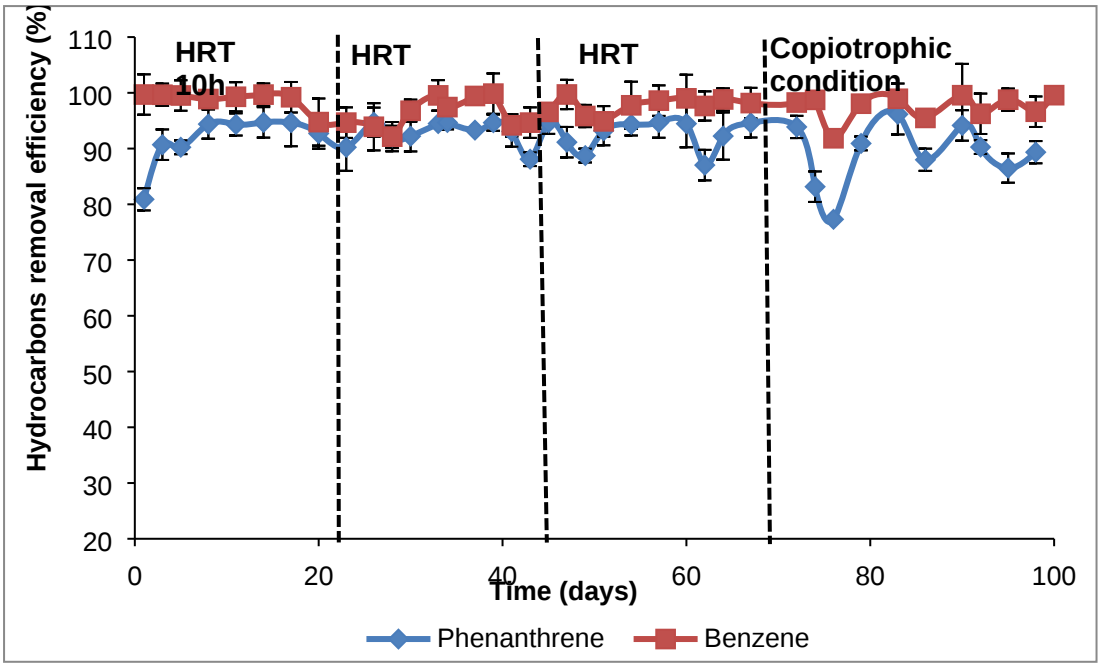


Fig. 9: COD removal and Hydrocarbons removal efficiencies at different HRTs and copiotrophic condition at ambient temperatures (15-25□) using adapted microbial consortia. Error bars ± SD are based on duplicate measurements.

As indicated in Fig. 10, high HRT was in favour of increasing power generation in air-breathing tubular MFC and the HRT of 30 h was found optimum for high MFC performance. The results of this study were similar other authors who have previously demonstrated that low HRTs adversely affected MFC performance (Huang and Logan, 2008; Rahimnejad et al., 2011; Li et al., 2013). Li et al (2013) studied the effect of HRT on MFC performance using animal carcass wastewater as anode feed in an up-flow tubular MFC. They found that maximum power density increased up to 2.19 Wm^{-3} when the HRT was switched from 3 to 5 d. The power outputs increase with longer HRTs as observed in this study and other previous studies could be due to the longer contact time between biofilms and organic matters in the anolyte, which would benefit biofilms to uptake, degrade substrates, and to transfer generated electrons onto anode surfaces (Sharma and Li, 2010).

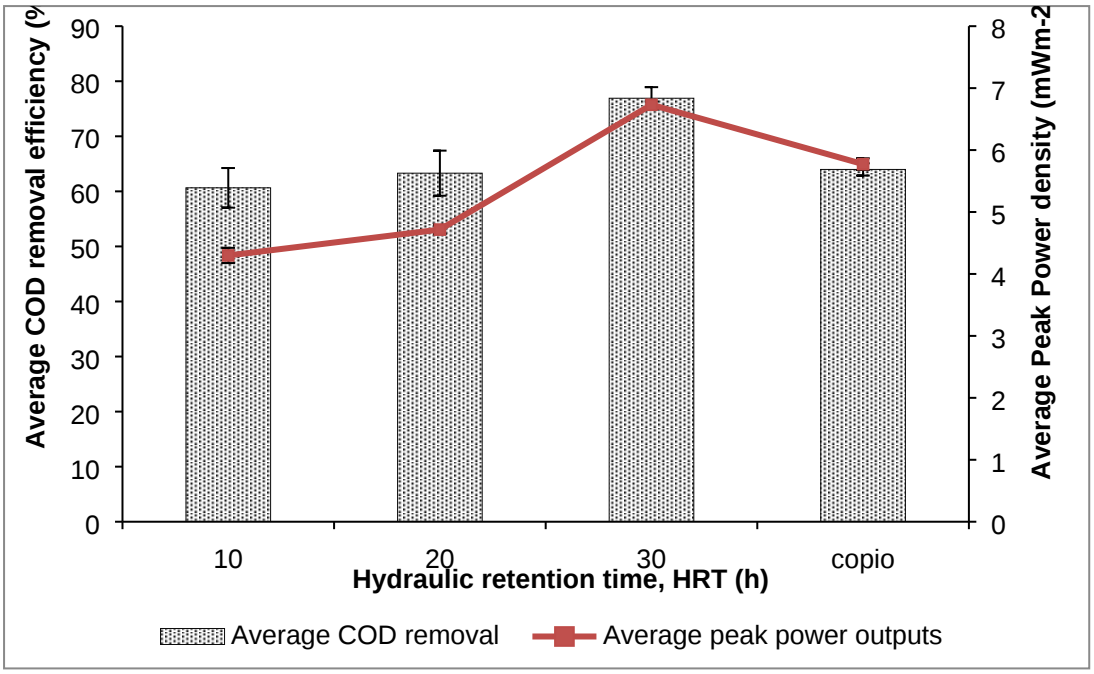


Fig. 10: Average COD removal efficiencies and peak power densities generated as a function of time at different treatment conditions in a novel tubular MFC system over 100 days period of continuous operation.

The results of this study imply that pollutants removal and power recovery could be maximized by increasing HRT to a preferred value. Therefore, it provided a possibility to treat high strength organic wastewater in future deployments.

At copiotrophic conditions (high organic shock loading), good MFC performance (i.e. COD removal, 64 % and power density, 5.78 mWm^{-2}) was obtained in the tubular MFC at HRT of 30 h. This clearly indicates that the MFC reactor had the ability to resist organic shock loading and maintain a stable performance under these test conditions; hence indicating the robustness of the reactor system. It has been reported that MFC may maintain its performance (in terms of COD removal) without any process inhibition (Fernando et al., 2014; Kraume et al., 2009).

The tubular MFC system was operated at ambient temperature and temperature fluctuations had little or no apparent effect on pollutant removal efficiency and power generation throughout the period of continuous MFC operation. Results suggest the potential use of MFC technology for posssible *ex situ* hydrocarbon-contaminated groundwater treatment or refinery effluents clean-up even at extreme (high contaminant levels -i.e. Total petroleum hydrocarbon,TPH, content above 1000 mg L^{-1}) condition with energy recovery as an added economic value. Electricity generated from these MFCs can be used to poised electrodes at a certain voltage for the degradation of other reclactricant compounds in MEC systems.

3.2.2 Ecotoxicological analysis

Discharge of wastewater is controlled by environmental regulation to ensure effluents do not have adverse environmental effects. Toxicity levels of the treated wastewater must be below a permissible levels and pose no risk to public health and the environment (Liu et al., 2010; Melo et al., 2013). Fig. 11 shows the evolution of inhibition on the growth of bioluminescent marine bacteria, *V. fischeri,* due to the direct contact with liquid samples of MFC effluents taken from

both operating conditions. Bioluminescence based acute toxicity assays conducted using *V. fischeri* indicated diminished of 61 % and 42 % in the toxicological level in MFCs operated at HRT 30 h and copiotrophic condition respectively. The observed decrease in toxicity level (as indicated by drop in bioluminescence inhibition of test microorganism) may be attributed to the transformation of the parent pollutants to less toxic metabolites.

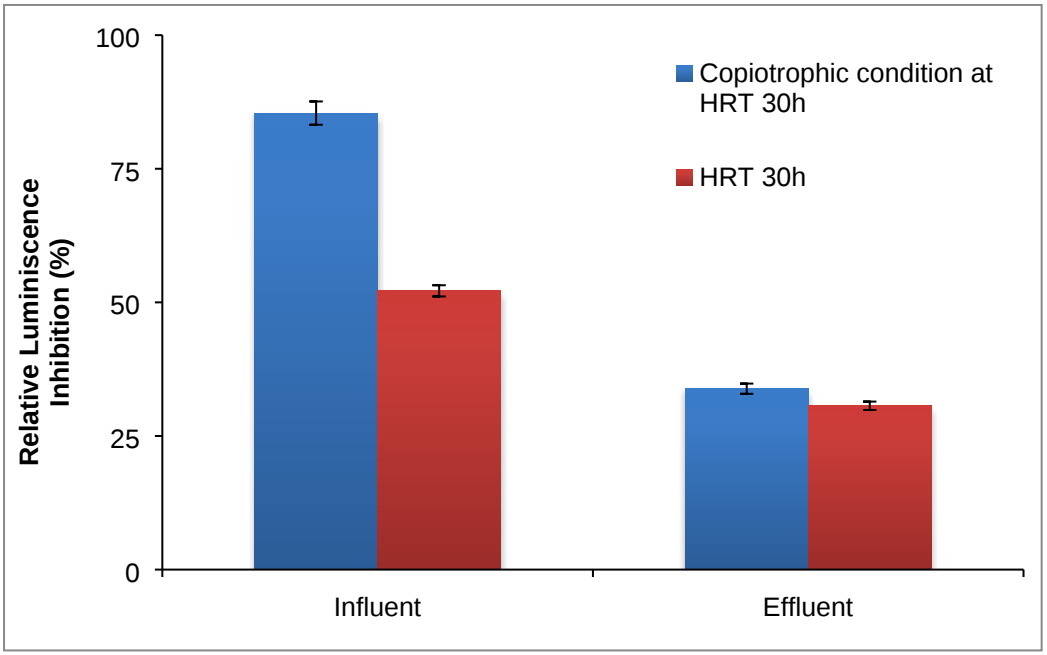


Fig. 11: Toxicity levels of MFC effluents at HRT 30 h and copiotrophic condition using adapted microbial consortia as inoculum source. Values are means of duplicate experiments ± SD.

The findings of this study further reinforce the evidence reported elsewhere (Rodrigo et al., 2014; Melo et al., 2013; Hamdi et al., 2007) on possible cytotoxic effects of post-treatment wastewater in anaerobic bioreactors such as well-stirred batch reactors, tubular or fluidized bed reactors. Ecotoxicology may provide a better insight into ecological assessment of remediation and may support decisions for safe discharge of treated wastewater to the environment (Hankard et al., 2004)

4. Concluding remarks

The findings of this study clearly demonstrated that HRT significantly influenced MFC performance at optimum operating conditions for both *in situ* and *ex situ* MFC deployments This suggests the application of MFCs in the simultaneous removal of petroleum hydrocarbons and bromate (with concomitant electricity generation) in anoxic environments, even at extreme conditions. MFC technology could possibly be a substitute for the more expensive conventional technologies such as permeable reactive barrier (PRB) and electroremediation.

Conflicts of interest

The authors hereby declare that they have no conflicts of interest associated with the information presented in this study.

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