Bioremediation of petroleum hydrocarbons using microbial fuel cells

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BIOREMEDIATION OF PETROLEUM HYDROCARBONS USING MICROBIAL FUEL CELLS

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Abstract

Environmental pollution by petroleum hydrocarbons has serious environmental consequences on critical natural resources upon which all living things (including mankind) largely depend. Microbial fuel cells (MFCs) could be employed in the treatment of these environmental pollutants with concomitant bioelectricity generation. Therefore, the overarching objective of this study was to develop an MFC system for the effective and efficient treatment of petroleum hydrocarbons in both liquid and particulate systems.

Biodegradation of target hydrocarbons, phenanthrene and benzene, was investigated in dual-chambered microbial fuel cells (MFCs) using different inoculum types - *Shewanella oneidensis* MR1 14063, *Pseudomonas aeruginosa* NCTC 10662, mixed cultures and their combinations thereof. All the inocula showed high potentials for phenanthrene and benzene degradation in liquid systems with a minimum degradation efficiency of 97 % and 86 % respectively with concomitant power production (up to 1.25 mWm^{-2}).

The performance of MFCs fed with a mixture of phenanthrene and benzene under various operating conditions - temperature, substrate concentration, addition of surfactants and cathodic electron acceptor type – was investigated. The interaction effects of three selected operating parameters - external resistance, salinity and redox mediator were also investigated using response surface methodology. The outcomes of this study demonstrated the robustness of MFCs with good degradation performance (range 80 - 98 %) and maximum power production up to 10 mWm-2 obtained at different treatment conditions. Interactive effects existed among the chosen independent factors with external resistance having a significant impact on MFC performance, with maximum power output of 24 mWm^{-2} obtained at optimised conditions - external resistance (69.80 kΩ) , redox mediator (29.30µM, Riboflavin) and salinity (1.3 % w/v NaCl).

The treatment of a mixture of phenanthrene and benzene using two different tubular MFCs designed for both *in situ* and *ex situ* applications in aqueous systems was investigated over long operational periods (up to 155 days). The outcomes of this work demonstrated stable MFC performance at harsh nutrient conditions and ambient temperatures. Simultaneous removal of petroleum hydrocarbons (> 90 %) and bromate, used as catholyte, (up to 79 %) with concomitant biogenic electricity generation (i.e. peak power density up to 6.75 mWm^{-2}) were observed.

The performance of a tubular MFC system in phenanthrene-contaminated soil was investigated in the last study. 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.

The overall outcomes of this study suggest the possible application of MFC technology in the effective treatment of petroleum hydrocarbons contaminated groundwater or industrial effluents and soil systems (mostly in subsurface environments), with concomitant energy recovery. MFC technology could potentially be utilised as an independent system in lieu of other bioremediation technologies (e.g. pump and treat, electrobioremediation or permeable reactive barriers) or integrated with existing infrastructure such as monitoring wells or piezometers.

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List of publications

Peer – reviewed journal articles

Adelaja, O., Keshavarz, T., Kyazze, G. (2015) The effect of salinity, redox mediators and temperature on anaerobic biodegradation of petroleum hydrocarbons in microbial fuel cells. *Journal of Hazardous Materials*, 283:211-217.

Adelaja, O., Keshavarz, T., Kyazze, G. (2014) Enhanced biodegradation of phenanthrene using different inoculum types in a microbial fuel cell. *Engineering in Life Sciences,*14(2):218-228.

Manuscripts ready for submission

Adelaja, O., Keshavarz, T., Kyazze, G. Treatment of petroleum hydrocarbons in a continuous operation using two different tubular MFCs.

Adelaja, O., Keshavarz, T., Kyazze, G. Enhanced bioelectrochemical remediation of phenanthrene-polluted soil using a dual chamber MFC.

Adelaja, O., Keshavarz, T., Kyazze, G. The effect of external resistance, surfactant and electron acceptors type on the performance of MFC fed with petroleum hydrocarbons.

Conference publications

Adelaja, O., Kyazze, G., Keshavarz, T. (2014) Effect of hydraulic retention time on the performance of a novel tubular MFC fed with petroleum hydrocarbons. *New Biotechnology*, 31 S98 1871-6784.

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Author's Declaration

I declare that the present work was carried out in accordance with the Guidelines and Regulations of the University of Westminster.

This thesis is entirely my own work and that where any material could be construed as the work of others, it is fully cited and referenced, and/or with appropriate acknowledgement given.

Until the outcome of the current application to the University of Westminster, the work will not be submitted for any such qualification at another university or similar institution.

Signed: Date: 30th July, 2015

Oluwaseun Adelaja

List of Abbreviations

AMC	Adapted mixed culture
ANOVA	Analysis of variance
APHA	American Public Health Association
AQDS	Anthraquinone-2, 6-disulfonic acid
AQS	Anthraquinone-2-sulfonate
BES	Bioelectrochemical system
BET	Brunauer, Emmett and Teller
BLAST	Basic Local Alignment Search Tool
BOD	Biochemical oxygen demand
BP	British Petroleum
BTEX	Benzene, Toluene, Ethyl benzene and Xylene
CCD	Centered composite design
CCFD	Centered composite face design
CE	Coulombic efficiency
CMC	Critical micelle concentration
CO	Co-culture of *S. oneidensis* and *P. aeruginosa*
COD	Chemical Oxygen Demand
CV	Cyclic voltammetry
DAF	Dissolved air floatation
DF	Dilution factor
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DS	Dry Soil
E^0	Standard reduction potential
E^0_{emf}	Electromotive force at standard conditions
E_{anode}	Anode potential
$E_{cathode}$	Cathode potential
E_{cell}	Cell voltage

EDTA	Ethylenediaminetetraacetic acid
EC_{50}	Half maximal effective concentration
E_{emf}	Electromotive force
EET	Extracellular electron transfer
EIS	Electrochemical impedance spectroscopy
F	Faraday's constant (9.64853×10^4 $Cmol^{-1}$)
FAS	Ferrous ammonium sulphate
FEPA	Federal Environmental Protection Agency
g	Gravitational force
HLB	Hydrophile-Lipophile Balance
HMW	High molecular weight
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic retention time
IARC	International Agency for Research on Cancer
IR_{Ω}	Sum of all Ohmic losses
ISCO	*In situ* Chemical Oxidation
LMW	Low Molecular Weight
LB	Luria-Bertani growth medium
MC	Anaerobic digested sludge
MCO	Anaerobic digested sludge with the co-culture
MCP	Anaerobic digested sludge with *P. aeruginosa*
MCS	Anaerobic digested sludge with *S. oneidensis*
MDCs	Microbial desalination cells
MECs	Microbial electrolysis cells
MFCs	Microbial fuel cells
MES	Microbial electrosynthesis
NAPLs	Non-Aqueous Phase Liquids
NCBI	National Center for Biotechnology Information
NCIMB	National Collection of Industrial and Marine Bacteria

NHE	Normal Hydrogen Electrode
NTA	Nitrilotriacetic acid
OCV	Open circuit voltage
OLR	Organic loading rate
ORR	Oxygen reduction reaction
P.A	*Pseudomonas aeruginosa*
PAHs	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
PDA	Photodiode array
P_{max}	Maximum power density
PEM	Proton exchange membrane
PHE	Phenanthrene
PRB	Permeable reactive barrier
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
R	Universal gas constant (8.314 J mol^{-1} K^{-1})
rDNA	Ribosomal DNA
rpm	Revolutions per minute
R_{ext}	External resistance
R_{int}	Internal resistance
ROI	Radius of influence
RSM	Response Surface Methodology
SD	Standard deviation
SHE	Standard hydrogen electrode
S.O	*Shewanella oneidensis*
SVE	Soil vapour extraction
T	Absolute temperature (Kelvins)
TAE	Tris- Acetate EDTA buffer
TCOD	Total chemical oxygen demand
TDS	Total dissolved solids
TEA	Terminal Electron Acceptor

TEMED	N',N,N,N' -tetra-methylethylenediamine
TPH	Total petroleum hydrocarbon
TU	Toxic unit
UASB	Upflow Anaerobic Sludge Blanket
UNEP	United Nation Environment Programme
USEPA	US Environmental Protection Agency
UASB	Up-flow anaerobic sludge blanket
UV	Ultraviolet
WHO	World Health Organisation
v/v	Volume per volume
w/v	Weight per volume
ΔG_r	Gibbs free energy (Joules)
$\sum \Delta_a$	Anode related overpotential
$\sum \Delta_b$	Cathode related overpotential
Π	Reaction quotient

CHAPTER 1:

GENERAL INTRODUCTION

1.0 Background

The petroleum industry has been one of the fastest growing industries over the past half century with a predicted increase in world petroleum consumption from 85 million barrels in 2006 to 106.6 million barrels by 2030 (Igunnu and Chen, 2012). The major raw material used in this industry is crude oil, a complex mixture of various hydrocarbon constituents. These constituents, generally called petroleum hydrocarbons, are widely used as fossil fuels and in the manufacture of numerous petrochemical products. They have contributed immensely to the global economy and industrialization. Unfortunately, the widespread use of petroleum hydrocarbons has increased pollution levels in the environment arising from leakages, indiscriminate disposal and accidental spills. According to Dadrasnia and Agamuthu (2013) between 1.7 and 8.8 million metric tonnes of oil were released annually into the global aquatic environment with more than 90 % being directly related to human activities. Over the past five decades, there have been several occurrences of significant oil spills into marine and terrestrial environments globally which are closely associated with anthropogenic activities. The recent BP oil spill in the Gulf of Mexico and the devastating effects of petroleum hydrocarbons in the Niger Delta, Nigeria (UNEP, 2011) are good examples of spillages from anthropogenic activities and the environmental impact they create (Figure 1.1). In Niger Delta area of Nigeria between 9 and 13 million barrels of oil seepage were reported over 2000 sites since oil exploration began with benzene and PAHs levels, 1800 and 500 times higher than WHO (World Health Organisation) standards respectively. The WHO standards for PAHs and benzene is 5 µg L^{-1} and 10 µg L^{-1} respectively. Two spills in Ogoniland required about $30 billion for clean-up operations over 30 years (UNEP, 2011).

Pollution from petroleum hydrocarbons in general results into acute deterioration of the marine, ground water and soil environments (Cervantes et al., 2011). Petroleum hydrocarbons can be bioaccumulated by plants and animals in soil and aquatic matrices via biomagnification along the food chains. If the concentration of pollutant is higher than its threshold concentration (i.e. the levels at which there are acute effects) then severe impairment of the metabolic activities or death of plants and/or animals can result (Johnson et al., 1996; Mandal et al., 2007). Some petroleum hydrocarbons (especially the aromatics such as benzene and phenanthrene) are carcinogenic and therefore listed among the EU priority pollutants (Hincapié et al., 2005). Hence, soil and groundwater pollution by petroleum hydrocarbons is an increasingly sensitive issue. In the European Union (EU) alone, there are about 3.5 million contaminated sites with an estimated cost of soil contamination in the range of $2.6-18.9 billion annually (Majone et al., 2015).

Figure 1.1: An oil-contaminated land site in Ogoniland area of Niger Delta region, Nigeria (UNEP, 2011).

While a number of approaches such as chemical oxidation, photo-oxidation, use of dispersants etc. have been employed in the cleaning up of hydrocarbon-contaminated sites (Zhang et al., 2010a); most of the processes are expensive, ineffective, too slow or environmentally unfriendly. Bioelectrochemical systems (BESs) have recently attracted much attention owing to their eco-friendliness, ambient operating temperatures with biologically compatible materials, versatility and production of useful products (e.g. electricity, fuels and chemicals). The use of bioelectrochemical systems (BES), an emerging technology, could be a sustainable and environmentally benign way of removal petroleum hydrocarbons from the environment with simultaneous power production.

This chapter introduces the focus of this thesis which is to design and investigate the use of bioelectrochemical system for the treatment of petroleum hydrocarbons in soil and aqueous systems in order to enhance biodegradation of petroleum hydrocarbons especially in sub-surface environments. This chapter also introduces general understanding of petroleum hydrocarbons and existing treatment technologies. The use of bioelectrochemical systems (BES) could be a cost-effective and eco-friendly way of treating oil contaminated sites and wastewater with concomitant energy recovery (Morris and Jin, 2012; Boghani et al., 2014).

1.1 Petroleum hydrocarbons in the environment: Sources and physico-chemical properties.

Over two centuries, petroleum has been reported to be found predominantly in surface soils, deep or shallow aquifers, aquitards, ocean sea-beds and oil seepage globally with the first oil exploration from an oil well in Pennsylvania in 1859 by

Colonel Drake (Alloway and Ayres, 1993). Subsequently, oil exploratory activities have increased exponentially worldwide over the decades with global continental reserves represented in Figure 1.2.

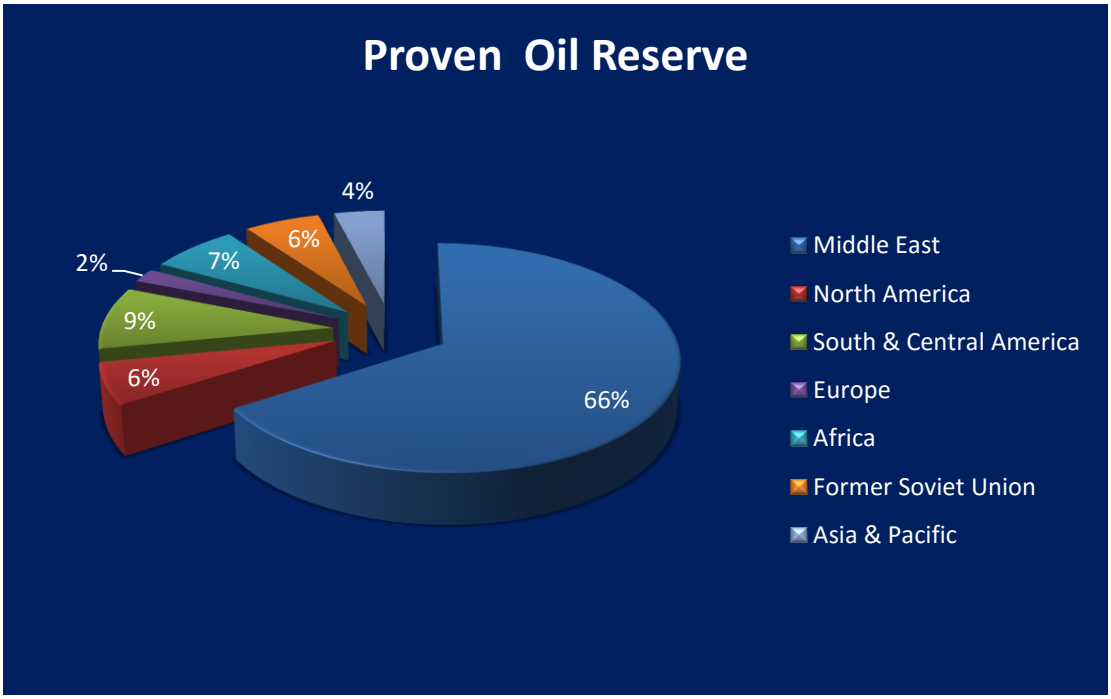


Figure 1.2: Proven oil reserve by continents (Image adapted from www.bp.com/world energy/).

Petroleum hydrocarbons are composed of a mixture of hydrocarbons obtained from reservoirs of crude petroleum which varies from simple aliphatic and aromatic compounds to complex, polycyclic aromatic and heterocyclic compounds (Figure 1.3). The proportion of the constituents in crude petroleum oil depends largely on the nature, geochemistry and types of oil reservoirs (Juana et al., 1998). The aliphatic hydrocarbons are saturated organic compounds with straight, branched or cyclic carbons atoms. Lighter crude oil contains higher proportion of aliphatic short-chain hydrocarbons while viscous heavy oil are composed of high proportion of aromatic hydrocarbons, mostly of high molecular weight compounds with rising degree of heterogeneity.

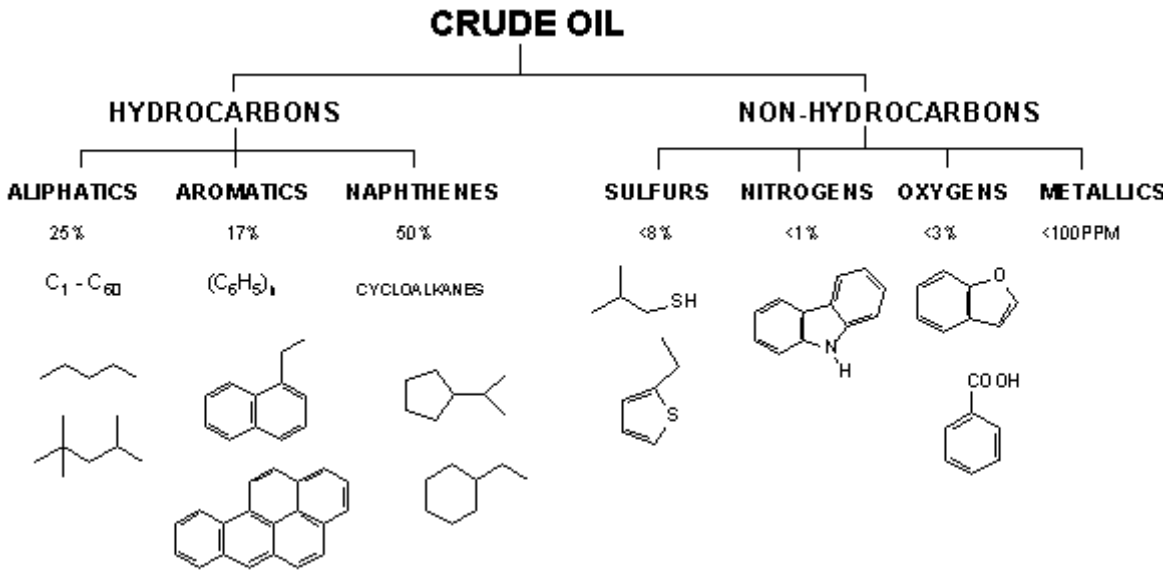


Figure 1.3: Average composition of crude oil along with some representatives of each class of compounds (Source- http://cann.scrantonfaculty.com/industrialchemistry/industrialchemistry module.html).

1.2 Classification of petroleum hydrocarbons

Petroleum hydrocarbons can be classified into three major classes: aliphatics, aromatics and heterocyclics.

1.2.1 Aliphatic hydrocarbons

Aliphatic hydrocarbons are straight, branched or cyclic compounds which may be either saturated or unsaturated. They can generally be subdivided into alkanes, alkenes and cycloalkanes. Alkanes (or paraffins) are saturated hydrocarbons with a general formula C_nH_{2n+2} and have carbon and hydrogen atoms linked together by single bonds only. They are either straight-chained or branched and form structural isomers which different physicochemical properties from straight-chain ones (Nathanail and Bardos, 2005). They are odourless, colourless and are of generally low reactivity though they can be easily oxidised by microorganisms at the terminal carbon position. Cycloalkanes (commonly known as naphthenes) are different from alkanes in that their carbon atoms are bonded together to form one or more ring-like

structures. Cycloalkanes share similar physical and chemical properties though cycloalkanes with only one ring are susceptible to nucleophilic attack, hence less stable and a little more reactive than normal alkanes (Mulligan, 2002). However, as the number of carbons and ring structure increases, they become more stable.

Alkenes or olefins are unsaturated hydrocarbons containing at least one carbon-carbon atom double bond. Alkenes also comprise straight-chain, branched-chain or cyclic configurations. Unlike alkanes and cycloalkanes, alkenes have shorter σ-bond length than alkanes and the presence of π-bonds makes alkenes more reactive chemically than alkanes and cycloalkanes (Patrick, 2000).

1.2.2 Heterocyclic hydrocarbons

Heterocyclic hydrocarbons are cyclic organic compounds containing at least two different elements as members of its rings. Examples of such elements include nitrogen, sulphur, oxygen and metals. The type of ring-like structure depends on the number of heteroatoms present in the ring and this varies from 3 to 8-membered ring compounds. Examples of heterocyclic compounds include pyrrole, dioxane, furan, pyridine, furfural etc. Depending on the type and nature of the heterocycles, they can be either be saturated or unsaturated. They are also very reactive and highly susceptible to both chemical and microbial attack (Nathanail and Bardos, 2005; Johnson et al., 2003).

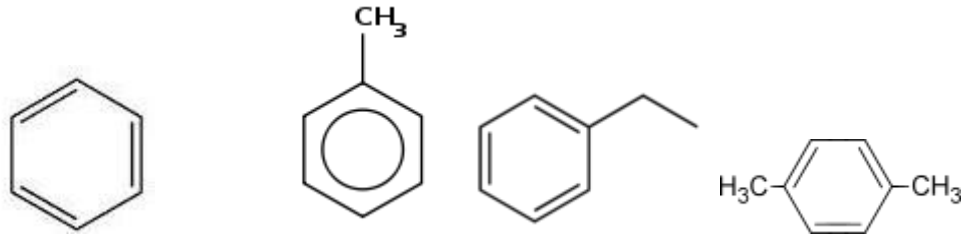
1.2.3 Aromatic hydrocarbons

Aromatic hydrocarbons differ significantly from aliphatic hydrocarbons due to the presence of ring structures solely. They are also unsaturated hydrocarbons due to the presence of two or more double bonds conjugated together to form a ring-like structure. The aromatics are the focus of regulatory agencies due to their persistent

and toxic effect in the environment. They are compounds that contain one or more benzene rings. They can be further divided into two categories namely; monoaromatics and polyaromatics.

1.2.3.1 Monoaromatics - BTEX

Benzene, toluene, ethyl benzene and xylene (BTEX) are aromatic hydrocarbons with only one parent benzene ring (Figure 1.4). They are components of gasoline, aviation fuels and other major petrochemical products. They are carcinogenic, neurotoxic and have been blacklisted by the Environmental protection Agency (EPA), UK (Juana et al., 1998; Foght, 2008). Contamination of groundwater with BTEX compounds is difficult to remedy because these compounds are moderately soluble in water (e.g. solubility of benzene in water = 1780 mg L^{-1} at room temperature) and can diffuse quickly once introduced into an aquifer (Lovanh et al., 2002).



Benzene Toluene Ethyl benzene p- xylene

Figure 1.4: BTEX compounds

Out of the BTEX compounds, benzene has been shown to be the most recalcitrant compound and highly mobile in matrix owing to higher solubility relative to other BTEX compounds (Johnson et al., 2003; Coates et al., 2002). WHO has established the maximum permissible level of benzene in water as 0.5 mg L^{-1} (USEPA, 2011a).

1.2.3.2 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are a class of fused ring aromatic compounds with the parent ring mainly of benzene. Examples of PAHs include naphthalene, fluorene, phenanthrene, anthracene, acenaphthene e.t.c (Figure 1.5). PAHs are naturally found in crude oil, coal deposits, marine sediment, soil and in groundwater (Juana et al., 1998). Anthropogenic sources include accidental spillage, improper disposal of petroleum products (creosote and coal tar), incomplete combustion of organic biomass and industrial discharges (Crawford and Crawford, 1996). They are lipophilic compounds with low aqueous solubility and thermodynamically unstable due to their relatively large negative resonance energies. Thus, they tend to be adsorbed on particles' surfaces in the environment or form non-aqueous phase liquids (NAPLs). As the molecular weight and number of fused rings of PAHs increases, the solubility and volatility decreases. In addition, they are generally persistent in the environment and have been reported to be carcinogenic (Johnson et al., 2003).

PAHs are recognized as a worldwide environmental contamination problem because of their intrinsic chemical stability, high resistance to various transformation processes and toxicity (Juana et al., 1998). As a result of the potency of PAHs in the environment, stringent legislations such as the EU directive 91/271/EEC on urban wastewater treatment and Federal Environmental Protection Agency (FEPA, Nigeria), have been enacted to ensure controlled PAHs' emissions into the environment via major point sources (Yan et al., 2004).

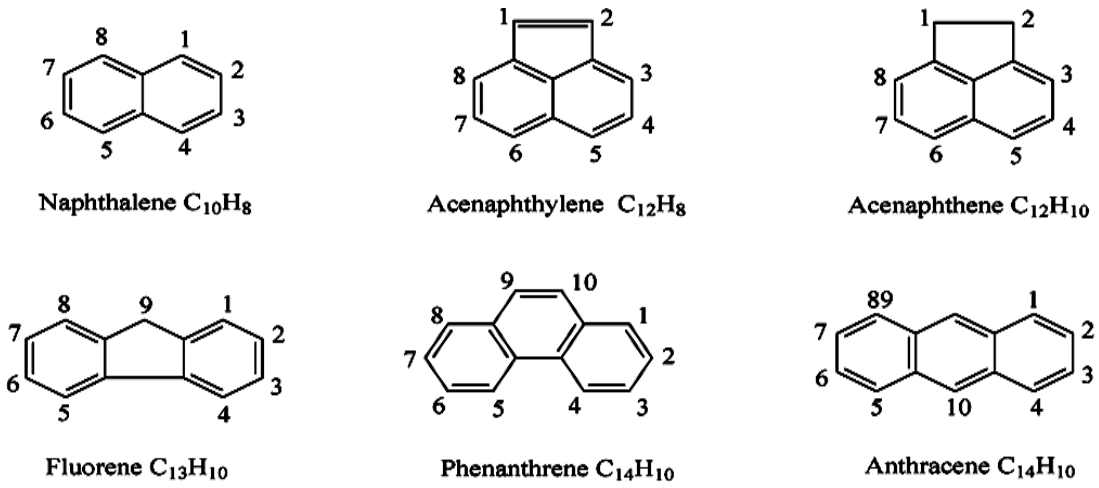


Figure 1.5: Structure of representative PAHs (Johnsen et al., 2005)

1.3 Existing methods for treating petroleum hydrocarbons

As a result of established risk to public health and the natural environments, treatment of petroleum hydrocarbons has increasingly become inevitable. However, a number of methods are currently used to remediate petroleum hydrocarbons, ranging from physico-chemical to biological methods. Each of these methods is briefly discussed below.

1.3.1 Physico-chemical methods

One of the long established physico-chemical methods of removing petroleum hydrocarbons from contaminated sites is the use of skimmers and booms. They are used to isolate oil spills and remove them from water (USEPA, 2011a). This is a very proactive, fast but capital intensive method and is mostly employed as an emergency response to oil spills especially in marine environment. A major disadvantage of this method is that this method does not treat or degrade the target pollutants but only removes them from the contaminated site for further treatment processing using other treatment technologies. In addition to this, it can only be applied on surface water.

Adsorption of petroleum hydrocarbon is another physico-chemical method that makes use of a range of adsorbents such as organoclay, activated carbon, copolymers, zeolite and woodchips. For example, activated carbon can remove dissolved petroleum hydrocarbons effectively (Fakhru'l-Razi et al., 2009). However, the disposal of spent adsorbents is a major challenge associated with adsorption methods. Some adsorbents such as zeolite, organoclay and copolymers are functionally specific in their applications. In addition, these materials are generally expensive and their regeneration processes are problematic and unsustainable (Frick et al., 1999; Lundstedt, 2003).

Thermal desorption is an innovative, non-destructive technology and proven physico-chemical method employed in the treatment of petroleum-contaminated soils. In this method, organic contaminants are converted from solid to gaseous phase by raising their vapour pressures through applied heat, hence separating the organics from the soil matrix (Riser-Robert, 1998). Incineration is also a physico-chemical method based on thermal destructive process in which petroleum-contaminated soil or refinery effluents are completely destroyed by application of heat at high temperatures. However, thermal treatment, such as thermal desorption, incineration and pyrolysis technologies are not widely employed for treating petroleum hydrocarbon-contaminated soil due to their high costs, high carbon footprint and toxic gases emission problems (Riser-Robert, 1998; Boesch et al., 2014).

Chemical methods involving the use of chemical reagents (e.g. dispersants) are employed in emulsifying hydrocarbons in oil-contaminated soil and water. However,

they are inefficient in dispersing heavy molecular weight hydrocarbons and have been reported to be toxic to the environment (USEPA, 2011b). Practically, they do not actually remove the pollutants but disperse them into the water body for degradation by other natural processes such as photodegradation and natural attenuation. Other chemicals e.g. hydrogen peroxide have been used for *in situ* chemical oxidation of contaminants. However, the reactions though fast are often short-lived, require multiple injections of peroxide and may produce undesirable byproducts preventing further degradation (Rico-Martínez et al., 2013). Johnson et al (1994) found that oxidized products (e.g. quinones, dihydrodiols and catechols) formed as a result of photodegradation are even more toxic than the parent pollutant and thus may inhibit microbial activity thereby lowering the degradation rates (Wen-xiang et al., 2007). Again, this method is also limited in application to surface matrices only and has high environmental risks due to high exposure to volatile organic compounds coupled with high remediation costs.

Chemical precipitation which involves the use of coagulants and/or flocculating agents e.g. calcite, spillsorb, lime, caustic soda, soda ash and sulfides. Coagulation and flocculation can be used to remove suspended and colloidal particles on petroleum-contaminated sites, but are ineffective for removal of dissolved components (Juana et al., 1998; Frick et al., 1999). FMA, an inorganic mixed metal (Fe, Mg and Al) polynuclear polymer, has a good coagulation, de-oiling and scale inhibition properties especially in high SS (Suspended Solids) levels with removal efficiency greater than 92 % (Fakhru'l-Razi et al., 2009; Zhou et al., 2000). Generation of sludge and increased concentration of metals in effluents are some of the major demerits of the chemical precipitation.

Other physico-chemical methods include electrodialysis, electrochemical processes, volatilizations, dissolved air precipitation (DAP), Fenton process among others. However, most of these methods are often expensive, technologically complex and lack public acceptance (Lundstedt, 2003)

1.3.2 Biological Methods

Due to challenges encountered from the above in field applications, biological methods e.g. bioremediation, have been sought as an alternative strategy. Bioremediation is an evolving technology that uses the metabolic potential of microorganisms to detoxify (e.g. through biotransformation) or clean up polluted environments. It is cost-effective and environmentally friendly (Chang et al., 2002; Milic et al., 2009; Hernández et al., 2011). Bioremediation can be employed for non-specific contaminants such as reduction of BOD (Biochemical Oxygen Demand) and odour from organic rich sediment or possibly tailored to treatment of specific pollutants such as metals or petroleum hydrocarbons (Chang et al., 2002). These merits in addition to low energy requirements make biological methods preferable over physico-chemical methods. Bioremediation plays an important role in the cleanup of soils, sediments and groundwater contaminated with hazardous organic chemicals.

To achieve an effective bioremediation strategy requires a holistic understanding of the interaction among microbial ecology, prevailing environmental conditions, structural and physico-chemical characteristics of the contaminants as shown in Figure 1.6 (Suthersan and Payne, 2005; Haritash and Kaushik, 2009).

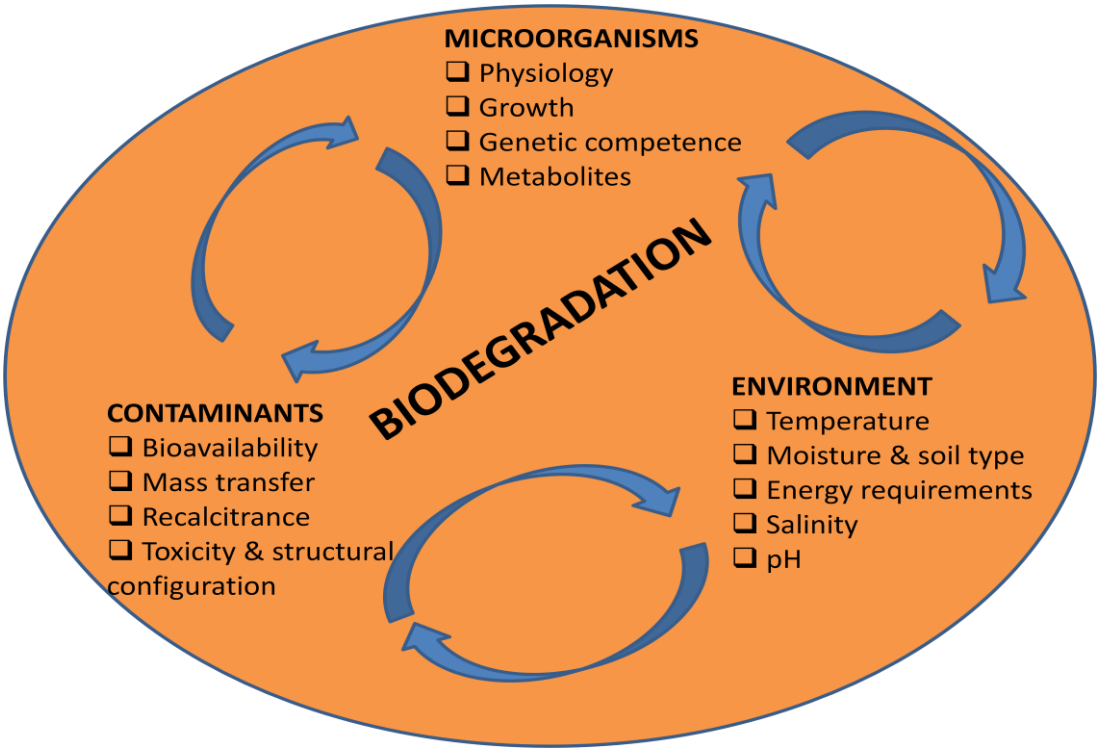


Figure 1.6: Biodegradation of contaminants in the environment: Their intrinsic interactions.

1.3.3 Microbial Bioremediation: Microorganisms involved in biodegradation process

Microorganisms including bacteria and fungi have been employed extensively in biodegradation of petroleum hydrocarbons (Johnsen et al., 2005). Non-biological degradation (physical transformation) such as adsorption, precipitation among others also occurs in the process; however, the biological transformation dominates the remediation process based on the growth of bacteria usually observed during the process (Helmy et al., 2009). While some species can only biotransform hydrocarbons (into intermediate metabolites), others can mineralize the hydrocarbons either aerobically or anaerobically (Johnsen et al., 2005). Due to the recalcitrant nature of most heavy and aromatic hydrocarbons, they are often degraded in the presence of a primary substrate -a process known as cometabolism (Luo et al., 2009). In cometabolism, the contaminant is reduced by an enzyme or

cofactor produced during microbial metabolism of another compound. Notably, biodegradation of the contaminant does not yield any energy or growth benefit for the microorganism mediating the cometabolic process reaction (Juana et al., 1998). Most known degraders have been reported to either utilise the contaminant as sole carbon source or degrade these compounds in the presence of another compound called co-substrate which may be readily oxidised by microbes for growth (Van Hamme et al., 2003; Mandri and Lin, 2007).

Due to the complexity and recalcitrance of some petroleum hydrocarbons, hardly will any one species of a particular genus be capable of fully degrading all range of petroleum hydrocarbons. Therefore, biodegradation of these compounds would usually require the mutual cooperation of more than one species (Riffaldi et al., 2006). *Pseudomonas* and *Acinetobacter* species are among the most common hydrocarbon–degraders reported in the literature (Bhattacharya et al., 2002; Pokethitiyook et al., 2003). Other typical bacterial groups already known for their capacity to degrade hydrocarbons include *Micrococcus, Vibrio, Brevibacterium, Corynebacterium, Flavobacterium, Marinobacter, Cellulomonas, Alcanivorax, Microbulifer* and *Sphingomonas* and some other species of proteobacteria and firmicutes groups (Nilanjana and Preethy, 2011; Nikolopouloua et al., 2007).

1.3.4 Factors affecting biodegradation rates of petroleum hydrocarbons

A number of limiting factors have been known to affect the rate of biodegradation of petroleum hydrocarbons as shown in Figure 1.7. The nature and physico-chemical properties of the petroleum hydrocarbon (i.e. contaminant) are one the foremost consideration among others factors when assessing the suitability of a remediation strategy. For instance, the lighter hydrocarbons (the aliphatics), due to their relatively

low molecular weight and structural complexity, have higher degradation rates than the aromatic hydrocarbons such as BTEX and PAH compounds. The addition of nutrients and improvement of other environmental factors such as pH, temperature and addition of co-substrate and surfactants have been extensively reported to enhance degradation rates (Yuodono et al., 2011; George-Okafor et al., 2009).

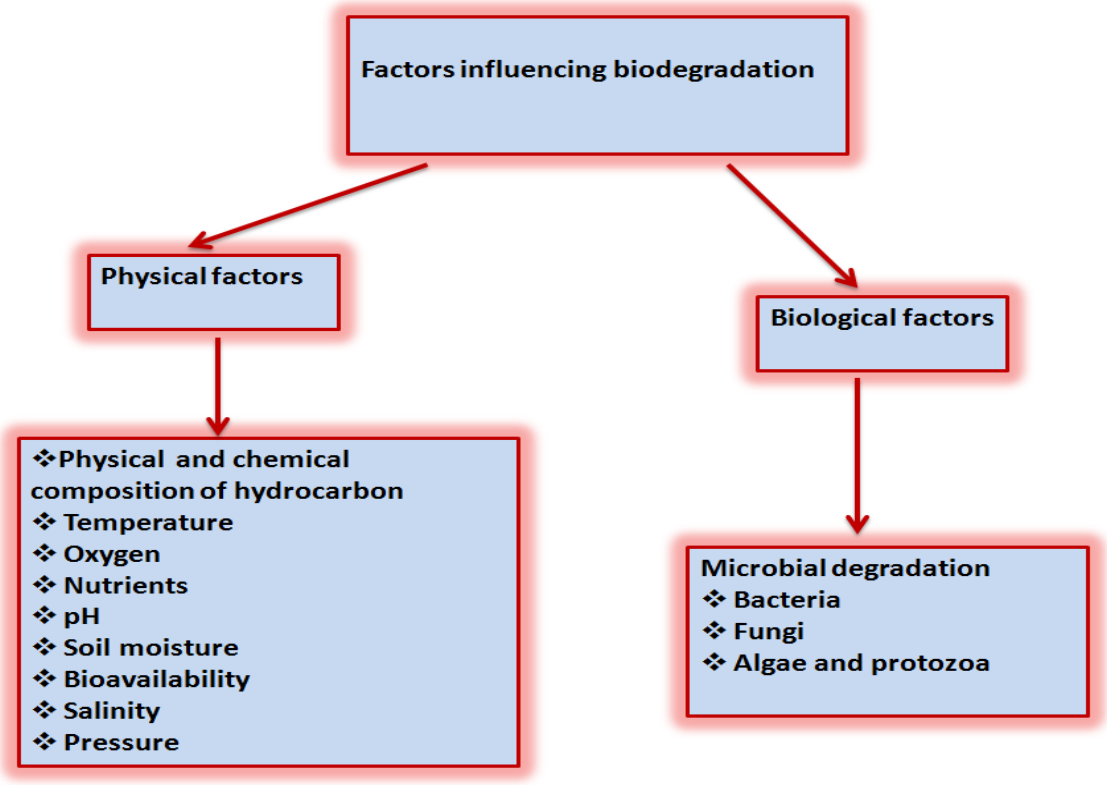


Figure 1.7: Environmental factors influencing the rate of biodegradation in the environment (Image adapted from Kriti and Chandra, 2014).

As molecular weight increases, water solubility decreases thus making hydrocarbons less biodegradable. Other factors such as the molecular structure & spatial arrangement, chemical reactivity, non-polarity, increasing number of aromatics rings, among others also contribute to the low degradability of PAHs (Johnson et al., 1994). PAHs, especially the high molecular weight (HMW)-PAHs are not easily degraded due to non-bioavailability of the substrate to microorganisms (Johnsen et al., 2005). Borden et al (1997) studied the biodegradation of BTEX compounds in a

contaminated site (under anaerobic conditions). They reported a degradation rate of 0.085 µM d^{-1} when microcosms were used in the treatment process (in lab experiments). In field experiments, under the same conditions, the degradation rate constant recorded was very low (0.014 µM d^{-1}). Low availability of nutrients (such as nitrogen, phosphorus and potassium, NPK) as previously mentioned may have contributed to the very low rates observed. Other degradation rates (at anaerobic conditions) reported include 0.06 µM d^{-1} for fluorene using sulphate as a terminal electron acceptor (TEA) (Coates et al., 1997) and 0.055 µM d^{-1} for naphthalene in an aquifer (Bregnard et al., 1996).

Microbial degradation of hydrocarbons depends largely on the availability of TEAs (terminal electron acceptors) e.g. O_2, NO_3^-, SO_4^{2-} (Foght, 2008). In anoxic environments (groundwater, soil and marine sediments), the availability of oxygen is very low or absent (Suflita and Caldwell, 2000). As a result of this constraint, biodegradation of hydrocarbons is often sustained by the continuous supply of nutrients like NO_3^- or SO_4^{2-} which serve as TEAs for microbial metabolism. This is another major limiting factor contributing to low degradation rates of hydrocarbon contaminants in soil and marine sediments (Foght, 2008; Johnsen et al., 2005). Several attempts like the supply of alternative TEAs to the soil or groundwater have met little success; for achieving complete mineralisation TEAs have to be continually fed into these matrices (Helmy et al., 2009; Olivera et al., 2009). The underlying technology is costly and unsustainable; therefore, it is not feasible for large-scale applications. Addition of nutrients like nitrates, phosphates and fertilizers which serve as biostimulants (for microbial growth) may have unintended environmental impacts e.g. eutrophication can lead to algal blooms in lakes, ponds or any other slow-moving water bodies (Nikolopouloua et al., 2007).

Temperature is an another important environmental factor which varies over a wide range (-10 to 55℃) and can directly influence biodegradation rates, the chemistry of the pollutant, physiology and the diversity of the microbial populations (Foght et al., 1996; Pelletier et al., 2004). Temperature also affects the solubility of petroleum hydrocarbons and degradation rates or microbial kinetics generally decreases with drop in temperature. Significant biodegradation of hydrocarbons however, have been reported in psychrophilic environments in temperate and Antarctica regions (Delille et al., 2004).

1.3.5 Mechanisms of petroleum hydrocarbons degradation by microbes

Biotransformation of BTEX and PAHs proceeds either by aerobic or anaerobic mechanisms (Figure 1.8). Aerobic transformation by BTEX and PAHs is achieved by ring oxidation mediated by enzymes (e.g. oxygenase) followed by ring cleavage (Johnsen et al., 2005). Low Molecular Weight (LMW)-PAHs can be biotransformed to trans-dihydrodiols by bacteria such as *Mycobacterium spp* using cytochrome P-450 monooxygenase (Kelley et al., 1990; Rockne et al., 1998). Fungi (e.g. White rot fungi) oxidize PAHs to quinones and phenolic derivatives by the action of peroxidase. The enzymes use molecular oxygen to oxidise these substrates leading to ring hydroxylation. Fungi tend to biodegrade most aromatic hydrocarbons at faster rates than bacteria (Johnsen et al., 2005). They form intermediate oxidised products that can be further oxidised by bacteria (Levina et al., 2003). However, fungi possess low competitive capabilities (especially when introduced into a natural ecosystem) and have longer acclimation times than bacteria (Gao et al., 2010). Petroleum hydrocarbon-degrading fungi species are also mainly limited to oxic and soil environments.

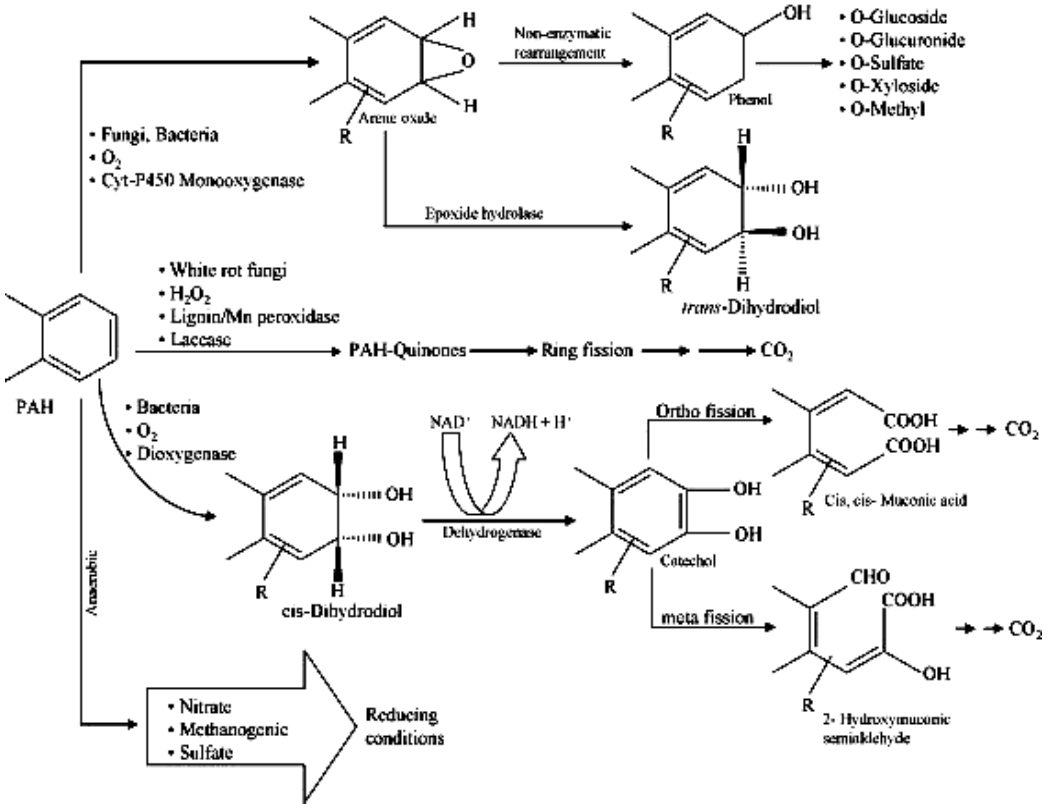


Figure 1.8: Proposed putative pathway for microbial catabolism of PAHs (Haritash and Kaushik, 2009).

Anaerobic degradation of PAHs and BTEX in the presence of TEAs (e.g. nitrates, sulphates and ferric ions) has been reported but the mechanism of degradation is not clearly understood (Meckenstock et al., 2004). Anaerobic biodegradation of phenanthrene has previously been reported to occur via carboxylation followed by cleavage of the aromatic ring putatively at the K region of the phenanthrene ring (Meckenstock et al., 2004). It is understood that anaerobic degradation pathway clearly differs from aerobic pathway. Tsai et al (2009) has proposed novel anaerobic biotransformation pathways of fluorene and phenanthrene by sulfate-reducing bacteria, SRB (Figure 1.9).

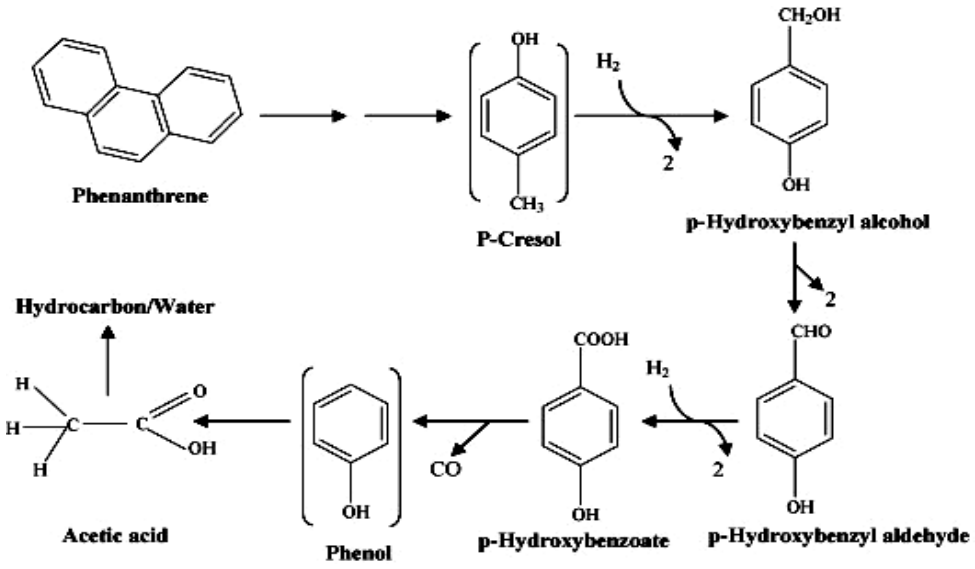


Figure 1.9: Proposed anaerobic biotransformation pathway of phenanthrene by sulfate-reducing bacteria (Tsai et al., 2009).

1.3.6 Bioremediation technologies

Bioremediation strategies can be grouped into two namely: intrinsic bioremediation or natural attenuation (NA) and engineered bioremediation. Intrinsic bioremediation is a do-nothing scenario in which the indigenous microbial population are allowed to degrade recalcitrant contaminants naturally without any engineered metabolic processes employed. The main disadvantage of this strategy is that it may take years to achieve good biodegradation efficiency (Kuiper et al., 2004). On the other hand, engineered bioremediation involves the use of various methods involving biostimulation, bioaugmentation or combined strategies in order to greatly enhance biodegradation efficiency by reducing the treatment period from many years to very few days depending on the extent and nature of the contaminated site (Talley and Sleeper, 1997; Taccari et al., 2012). This can be through *in situ* or *ex situ* treatment methods. Although they are more efficient and faster than natural attenuation, they are capital intensive and are often associated with different forms of environmental risks.

1.3.6.1 *Ex situ and in situ* bioremediation technologies

Bioremediation technologies can be broadly classified as *ex situ* and *in situ* bioremediation. ***In situ* bioremediation** technologies involve treatment of the contaminated material in its original location. It is widely applied to contaminated sites with relatively high depths, widely spread throughout the site (large area) and of low to medium contaminant concentration (Evans and Furlong, 2003). The fundamental basis of *in situ* bioremediation involves the injection of nutrients and electron acceptors to contaminated sites by various techniques with the main goal of inducing microbial activity in order to enhance biodegradation.

In contrast, ***ex situ* bioremediation technologies** are those treatments which involve the physical removal/excavation of the contaminated material from its original location to a treatment facility or another site for further treatment. These treatments are easily controlled and monitored and require less treatment period; hence they are more effective than *in situ* technologies. Most organic pollutants are strongly adsorbed in the soil matrix and difficult to access by *in situ* techniques, which coupled with time limitation, prompt the use of *ex situ* remediation techniques. However excavations, transport cost, environmental exposure risk during transport among others make it less cost effective. Some of the examples of *in situ* and *ex situ* bioremediation are given below:

Land farming is a process in which contaminated soil is spread out on thin layers (no more than 1.5m thick) and aerated by tilling the soil periodically with the addition of nutrients in order to promote indigenous contaminant degrading species. It is a solid-phase treatment system for contaminated soils where contaminants are

biodegraded and immobilised by microbiological processes and oxidation which may be done *in situ* or *ex situ* (Frick et al., 1999; Khan et al., 2004).

Composting: Composting technology involves the mixing of contaminated soil with a mature, cured organic material (called compost) to degrade target contaminants (Figure 1.10). The use of composted organic matter such as bark compost, spent mushroom and fishbone compost enriched with urea. They can provide supplemental nutrients, carbon source as well as microorganisms with high metabolic capability in the biodegradation of hazardous organic compounds present within the soil matrix (Antizar-Ladislao et al., 2006; Sayara et al., 2011).

Figure 1.10: Composting by windrows (Source-https://commons.wikimedia.org/wiki/File:Compost_site_germany.JPG).

Bioslurry system (Bioreactors): Enhanced biodegradation in a container or reactor is achieved by increasing the contact time between the contaminated soil and micro-organisms at optimum conditions of nutrients, oxygen and pH. It is widely used to treat industrial effluents and contaminated soils (Cassidy and Hudak, 2002).

Biopiles: Biopiles is another bioremediation technology that involves the stimulation of microbial activity through aeration and addition of nutrients in an excavated contaminated soil which have been piled into heaps. A typical biopiles can be up to 6m in height. Biopiles are aerated by injecting air under pressure through perforated pipelines placed throughout the pile (Khan et al., 2004).

Bioventing and biosparging: Bioventing is an *in situ* remediation technology in which indigenous microbial activity is enhanced by the injection of air at low rates (to prevent volatilisation) into the contaminated soil at the unsaturated zone via the use of injection wells (Frick et al., 1999). At times, when necessary, nutrients can also be added to stimulate the growth of the indigenous microbial population. Depending on the depth and site geology, constructed bioventing wells can be installed either horizontally or vertically.

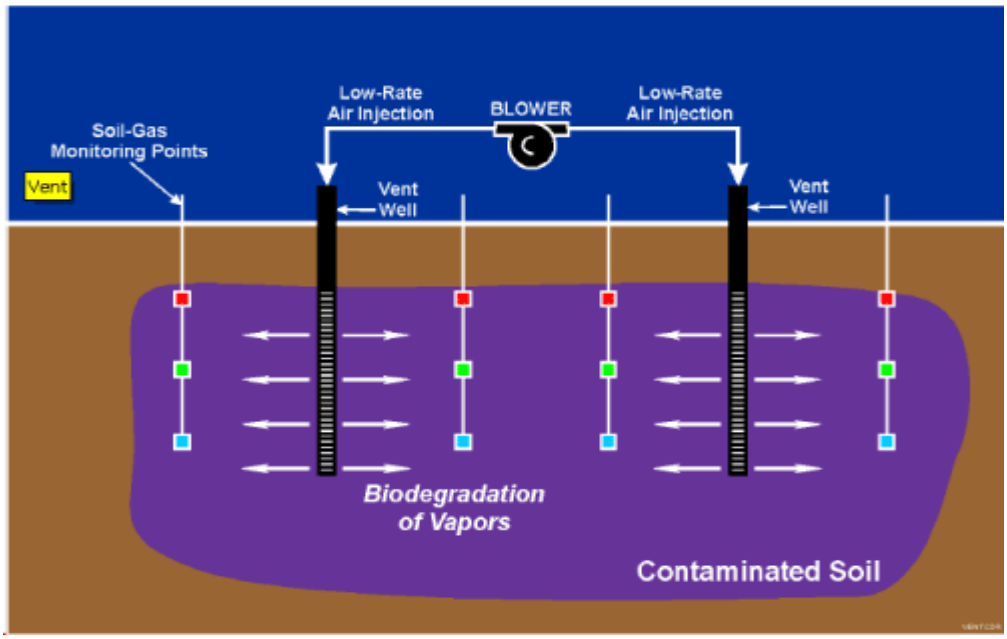


Figure 1.11: A typical representative design for bioventing and biosparging systems (Garima and Singh, 2014).

Biosparging systems are very similar in design and working principle to bioventing but differ mainly in operational zone; biosparging are used in saturated zone just below the groundwater level (Figure 1.11). Also, air injection flow rates are higher than bioventing systems. In real application, bioventing and biosparging systems are often combined with other remediation technologies such as soil vapour extraction (SVE), pump 'n' treat and permeable reactive barrier (PRB) technologies (Suthersan et al., 2005; Khan et al., 2004).

Permeable Reactive Barriers (PRBs): A permeable reactive barrier is an emplacement of permanent or replaceable reactive media in the subsurface across the flow path of a contaminated groundwater plume, typically under its natural gradient, thereby transforming the contaminants into less harmful and environmentally acceptable forms (Thiruvenkatachari et al., 2008). They are designed in such a way that contaminants are treated within the reactive barrier as groundwater flows readily through with minimal alteration to groundwater hydrogeology (Figure 1.12).

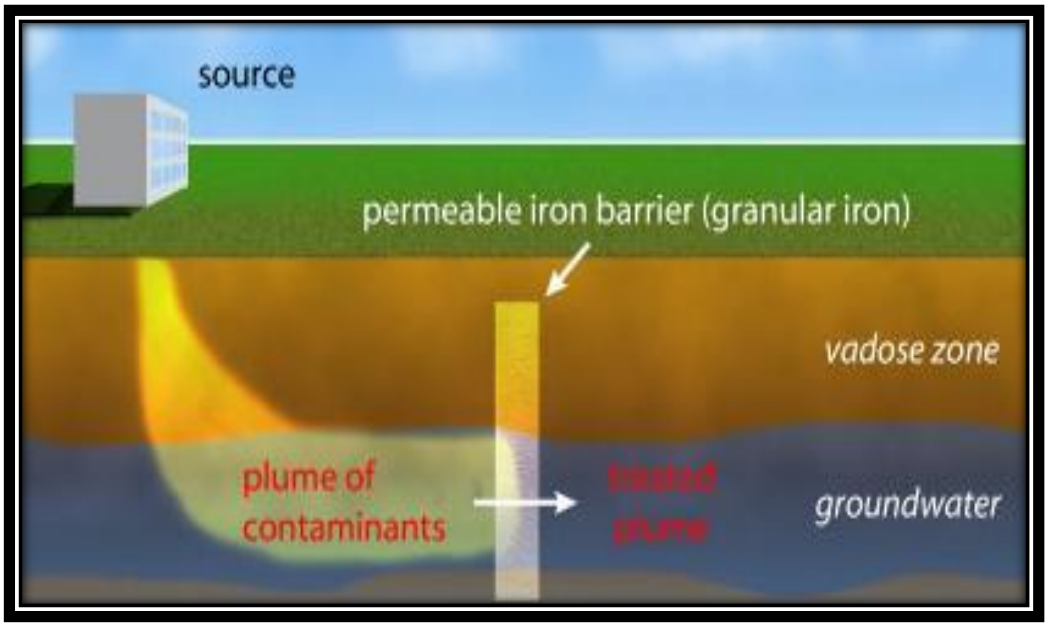


Figure 1.12: A typical conceptual design of a permeable reactive barrier (PRB) technology. (Source- https://commons.wikimedia.org/wiki/File:Iron_Wall_PRB.jpg).

Some of the advantages of PRBs over other conventional groundwater remediation technologies include low maintenance cost, *in situ* treatment, absence of above the ground facilities and groundwater reinjection. However, PRBs are only applicable to shallow aquifers and are also relatively costly in terms of capital cost (Scherer et al., 2000; Choi et al., 2007).

Electrobioremediation (Electrokinetic bioremediation): Electrokinetic bioremediation technology is a technique that utilises either electroosmosis and electromigration to initiate or enhance the *in situ* biodegradation of contaminants under a low voltage applied (3-20V) through electrodes placed within the contaminated soil (Figure 1.13). This technology is particularly effective in contaminated soil with low hydraulic conductivity and large surface area (Yueng et al., 2011). Biological growth factors such as surfactants, nutrients and microbial population can be transported at different orientations within the soil and groundwater matrices (Gan et al., 2009). Despite the efficacy of this technology, it has several limitations such as energy input, restricted applications to soils with low hydraulic conductivity, heterogeneity of the soil matrix and availability of the contaminants (Megharaj et al., 2011).

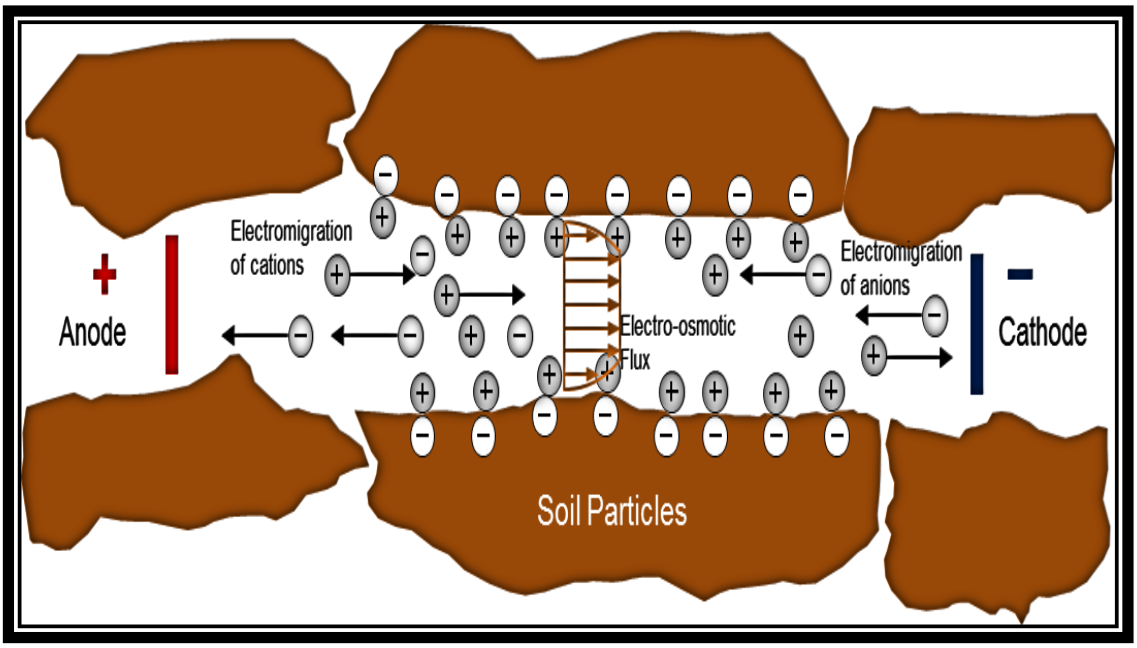


Figure 1.13: Electrokinetic bioremediation (Claudio et al., 2013).

Despite the advantages of biological methods, there are still several limitations such as substrate non-bioavailability, capital & maintenance costs, unsustainable and/or lack of terminal electron acceptors, toxicity of contaminants & their partially degraded products and lower biokinetics. Hence, there is a need to seek more efficient and sustainable approaches for the removal of hydrocarbon contaminants from the environment.

1.4 Bioelectrochemical systems (BESs) and its possible applications for petroleum hydrocarbon removal

Given the limitations associated with the traditional methods of treating petroleum hydrocarbons, there is a need for more cost-effective, efficient and eco-friendly methods for treating petroleum hydrocarbon contaminated sites and refinery wastewater. Recently, bioelectrochemical systems have been suggested as a potentially viable way of oxidizing various waste organics with concomitant electricity production (Rozendal et al., 2008; Hawkes et al., 2010).

1.4.1 Bioelectrochemical systems: Types and its brief historical background

Bioelectrochemical systems are unique and emerging set of technologies which use microbes attached to one or both electrode(s) to catalyze redox reactions in electrochemical cells (Rabaey et al., 2009). There are two main types of BESs which are commonly known as: Microbial Fuel Cells (MFCs) and Microbial Electrolysis cells (MECs). A BES is called a microbial fuel cell if the overall reaction is thermodynamically spontaneous and electric current is generated. Conversely, it is regarded to be a microbial electrolysis cell if electrical energy is supplied to drive a non-spontaneous electrochemical reaction.

MFCs can utilize microorganisms e.g. *Shewanella spp, Geobacter spp, Pseudomonas spp* and *Rhodoferax,* either in axenic or mixed microbial populations, to catalyze the anodic oxidation of substrates with contaminant biogenic electricity generation when connected to a load /resistor via an external circuit to the cathode. A reduction of protons to form water takes place at the cathode. The electric current generated in BESs is an indicative measure of substrate conversion /biodegradability of the organic substrate at the anode (Rabaey et al., 2009). MFC systems are novel and innovative technologies which have extensively been utilised in the wastewater treatment from different sources with renewable power generation (Mohanakrishna et al., 2010; Liu et al., 2004a; Patil et al., 2009). Other MFC applications include bioremediation of xenobiotics compounds, production of precious metals and valuable chemicals, and as biosensors (Luo et al., 2009; Zhang et al., 2010b; Kim et al., 2003).

MECs are typical electrolytic cells very similar to MFCs but with a slight modification; instead of an external load (as in MFCs), they utilize a small external power source to make the bioprocess thermodynamically feasible (Gibb's Free

energy, ΔG is negative) for redox reactions occurring in the anode and cathode (Figure 1.14B). For example, the anode could be poised with a small voltage (*ca.* 200 mV) to produce the required redox potential for spontaneous cathodic bio-hydrogen production (Wang et al., 2012a). MECs are also utilised in the production of many chemicals such as ethanol (Steinbusch et al., 2010), sodium hydroxide (Chen et al., 2012), hydrogen peroxide (Rabaey and Rozendal, 2010), microbial-assisted metal nanoparticles production and degradation of some chlorinated hydrocarbons (Bin et al., 2013; Wu et al., 2011).

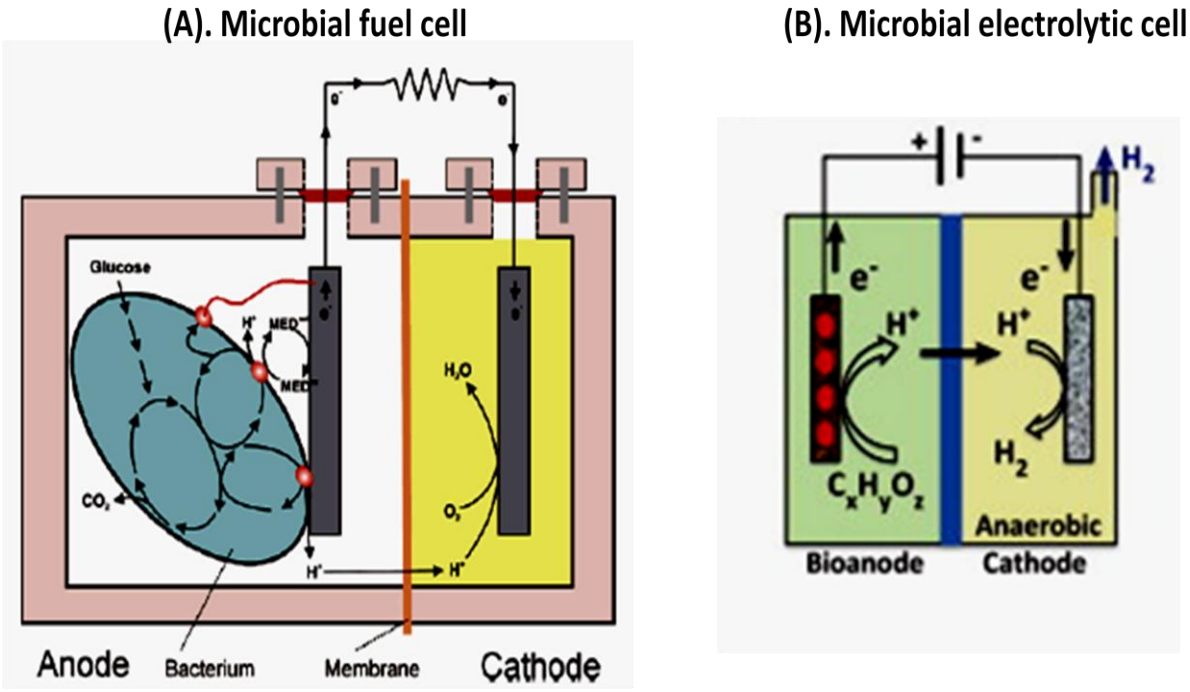


Figure 1.14: The operating principle of a microbial fuel cell (MFC) and microbial electrolysis cell (MEC) (images adapted from Han et al., 2013 and Logan et al., 2006)

While limited space prohibits a detailed historical evolution of BES, suffice it is to say that the origin of bioelectrochemical cells began in early 1900s by Potter M.C who demonstrated the generation of electric current in an electrochemical cell using a pure culture of *Escherichia coli* as biocatalyst (Potter, 1911). Subsequent to Potter's interesting contributions to BES studies, the concept of electrochemical

phenomena involving microbes has undergone a very slow evolutionary development over many decades with some minor contributions by Cohen (1931), Berk et al (1964), Suzuki et al (1978) and lastly, Allen and Benneto (1993). Not until early 1990s, as demands for renewable sources of energy intensified due to the rising global energy demands and its negative effect on climate change (resulting from over dependence on fossil fuels among other factors), did research activities into MFCs and other BESs increased. This led to significant technological development in BESs in terms of bioenergy generation, production of value-added chemicals and numerous useful applications. In tandem, research on types of reactor design, cathodic configurations and their compositions have also undergone technological advances. MFCs unlike MECs can be employed in the removal of recalcitrant contaminants coupled with energy recovery by converting stored chemical energy in the contaminants to usable bioelectricity. In view of this, MFC systems as one type of BES will be the focus of subsequent discourse throughout this thesis.

1.4.2 Working principle of MFCs

The working principle of MFC can generally be described as a typical electrochemical cell which consists of an anaerobic anode chamber and a cathode chamber physically separated by an ion selective membrane but connected to an external circuit over a load (Figure 1.14A). In the anode chamber, microorganisms oxidise organic substrates (such as glucose) into biomass with the release of electrons and protons. Following microbial oxidation, the protons produced migrate into the cathode chamber through the ion-selective permeable membrane. Meanwhile, the released electrons the microorganism are collected by the anode electrode and subsequently flow through an external circuit into the cathode where a chemical species with a highly positive redox potential such as oxygen, ferricyanide or other

oxidising chemical agents accepts the electrons and undergo a reduction reaction. Thus electricity is generated by this microbially-catalysed electrochemical cell. The redox reactions occurring in both chambers are given as follows:

Anodic half-cell reaction: $C_6H_{12}O_6 + 6H_2O \rightarrow 24e^- + 24H^+ + 6CO_2$.....(1)

Cathodic half-cell reaction: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ .....(2)

Overall redox reaction of the cell: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Energy$ .....(3)

The efficiency of the cathodic reaction which determines the overall system performance is dependent on many factors such as concentration and nature of species of the electron acceptor, proton availability and catalyst performance. One of the important factors that dictate cathodic performance in MFCs is the catalyst performance. In order to make the redox reaction spontaneous and electron flow across the cell through an external circuit to the cathode where other electron acceptors or oxygen reduction reaction (ORR) need to be catalysed using various ORR catalyst. The most commonly used cathodic catalyst in MFCs (as well as in conventional fuel cells) is platinum, Pt. In MFCs, the cathode which is usually made up of carbon material is coated with small quantity of Pt powder. However, the use of alternative cheaper catalyst material are being considered due to high cost of Pt. Examples of alternative catalyst previously reported include manganese oxide (Rhoads et al., 2005), iron and cobalt complexes (Cheng et al., 2006; Zhao et al., 2006), use of activated carbon or biochar (Hao Yu et al., 2007), biological catalyst materials such as laccases (Schaetzle et al., 2009) and electrochemically-active bacteria, algae and mixed microbial populations can also be used as biocathodes (Clauwaert et al., 2007; He and Angenent, 2006).

Proton migration through the ion-selective permeable membrane is a key factor determining MFC performance in terms of power generation. Poor proton transfer could results into high internal resistance (Logan et al., 2006). The efficiency of proton transfer in turn depends mainly on the nature and the properties of the membrane. Nafion membrane and cation-exchange membrane (CEM) are most commonly used proton exchange membranes in MFCs with Nafion being the most efficient but expensive material. High migration of other cation species (produced from the anode) into the cathode could result in an increase in pH and decrease in cathode potential for ORR due to increasing over potential build-up around the catholyte-electrode surface interface (Rozendal et al., 2006).

1.4.3 Electron transfer mechanisms by electrochemically-active microorganism

Electrons released during microbial oxidation within the cell are transported extracellularly to the anode. This occurs either through direct or indirect electron transfer mechanisms (Figure 1.15). The actual transfer mechanisms of electrons to a conducting electrode (anode) are poorly understood. Direct electron transfer to the anode have been proposed to occur through chains of microbial outer membrane-bound electron transfer proteins such as *c*-type cytochromes and an electrically conductive structure known as pili or "nanowire" (Rabaey et al., 2004; Kim et al., 2007). Electron transfer could be mediated through various soluble diffusible electron shuttles or redox mediators, which can be produced by the bacteria themselves endogenously or added exogenously. Examples of redox mediators (natural or synthetic) that have been reported in previous studies include soluble compounds such as riboflavin (Dos Santos et al., 2004), phenanize, pycocyanin, humic acids (Ma et al., 2011), anthraquinone-2, 6-disulfonic acid (AQDS) (Dos Santos et al., 2004),

neutral red (Park and Zeikus, 2000) and anthraquinone-2-sulfonic acid (AQS) (Rau et al., 2002).

A diverse range of microbial species used as biocatalyst in the anode of MFC consist of electrogenic and non-electrogenic microorganisms (Bonanni et al., 2013; Leung et al., 2013). Electrogenic microorganisms are bacterial species that can extracellularly transfer electrons to the anode; they are also known as anodophilic microbes (Shi et al., 2007). Various species of electrogenic bacteria have been recognized so far, including *Geobacter* species (Bonanni et al., 2013), *Shewanella* species, *Rhodoferax ferrrireducens* (Chaudhuri and Lovley, 2003), *Escherichia coli*, *Pseudomonas* species and most species in the phyla of proteobacteria and firmicutes (Rabaey et al., 2004; Park and Zeikus, 2003).

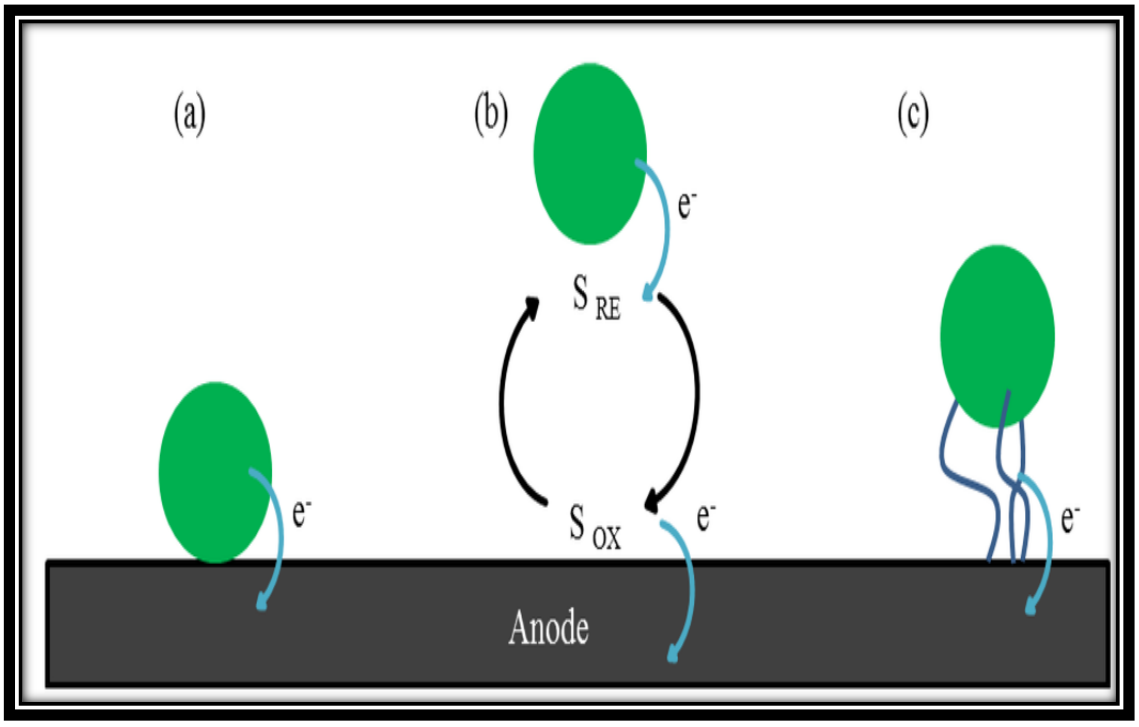


Figure 1.15: Extracellular electron transfer mechanisms: Microbially catalysed electron transfer from substrate reduction/oxidation onto electrode surface. This may occurs either through direct contact, mobile electron shuttles or nanowires respectively, as shown above (Chouler and Lorenzo, 2015).

However, there are also other groups of heterotrophic microorganism (mainly fermentative bacteria and methanogens) which do not contribute to electricity production but can collaboratively metabolise complex organic substrates into simpler metabolites that can be more directly assimilated by electrogenic bacteria in a syntrophic process (Venkataraman et al. 2011; Verstraete et al., 2007).

MFC performance relies on operating conditions such as the anode potential, type of substrate, environmental factors, applied voltage, external and internal resistance of the cell among others things. These factors have significant influence on the choice of biocatalyst and composition of microbial community present in MFCs. For instance, when the anode potential is very low and the concentration of redox mediators are low in the anode chamber, fermentation processes dominates electrogenesis where electrons are transferred internally in the bacteria leading to low electricity generation in MFCs (Rosenbaum et al., 2011; Yates et al., 2012).

1.4.4 Electrochemical aspects of MFCs

1.4.4.1 Thermodynamics and energy losses in MFC system

In MFCs, there must be a potential difference across the terminals in an electrochemical cell to initiate the driving force for flow of electrons. This is known as the cell electromotive force, E_{emf}. The Gibbs free energy is closely related to the cell emf, as it is the amount of energy available to drive the flow of electrons round an external circuit. Gibbs free energy and the cell emf are important parameters assessing the electrochemical performance of MFCs. The relation between the Gibbs free energy and cell emf is given as:

$$E_{emf} = - \Delta G_r / nF \quad \text{.....(4)}$$

Where, n is the number of electrons transferred per reaction and F is the Faraday's constant (9.64853 X 10^4 $Cmol^{-1}$) and ΔG_r measured in Joules (J) is the Gibb free energy for specific conditions which is expressed as:

$\Delta G_r = \Delta G^0_r + RT \ln\Pi$ .....(5)

where ΔG^0_r (J) is the Gibbs free energy under standard conditions (298.15 K, 1bar pressure, and 1M concentration for all chemical species), *R* is the universal gas constant (8.314 J mol^{-1} K^{-1}), *T* (K) is the absolute temperature and Π is the reaction quotient or equilibrium constant.

Under standard conditions, the cell emf can be written as follows:

$E^0_{emf} = - \Delta G^0_r / nF$ .....(6)

where, E^0_{emf} is the cell emf at standard conditions.

From equation (4-6), the cell emf for the overall reaction could be expressed as:

$E_{emf} = E^0_{emf} – (RT/nF) \ln(\Pi)$ ..... (7)

The E_{emf} of a MFC system can also be expressed as the difference in the anode and cathode potentials:

$E_{emf} = E_{cathode} – E_{anode}$ ..... (8)

The values of the electrode potential at both half-cells depend on the substrate oxidation and cathodic reduction reaction occurring in a typical MFC. For example, when 5mM of acetate (at pH 7) is used as sole substrate in a MFC with platinised cathode (Logan et al., 2006), the half-cell reactions are given as follows:

Half -cell reactions:

At Anode: $2HCO_3^- + 9H^+ + 8e^- \rightarrow CH3COO^- + 4H_2O$ (E_{anode} = -0.296 V).....(9)

At the Cathode: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ ($E_{cathode}$ = 0.805 V) .....(10)

On substituting individual electrode potentials in equation (8), E_{emf} of this MFC would be 0.805 – (-) 0.296 = 1.106 V

The open circuit voltage (OCV) is measured when no current is flowing or at infinite/zero resistance (Bard and Faulkner, 2001). Therefore in an ideal MFC, OCV would be much less than or equal to the theoretical (maximum) E_{emf} value obtainable (based on the anode and cathode potentials) and both OCV and E_{emf} can never exceed 1.1 volts. The OCV is considerable lower than the thermodynamic E_{emf} due to a number of internal losses within the electrochemical cell and the bulk electrolyte solution and at both terminals (anode and cathode). This voltage loss is known as overpotential or overvoltage. These internal losses are inherent limitations within a BES that need to be overcome to achieve improved energy efficiency. Considering the internal losses, the observed voltage or cell voltage, E_{cell} can be expressed as:

$$E_{cell} = E_{emf} - (\sum\Delta_a + \sum\Delta_b + IR_\Omega) \quad \text{.....(11)}$$

where $\sum\Delta_a$ and $\sum\Delta_b$ are the overpotentials of the anode and the cathode respectively, and IR_Ω is the sum of all ohmic losses which are proportional to the generated current by the BES.

The current dependent overpotentials in MFCs can be divided into activation losses, bacterial metabolic losses and mass transport or concentration losses (Logan et al., 2006).

Ohmic losses in MFCs arise from resistance to movement of electrons and ions through electrodes, electrode interconnections and the external circuit and the flow of protons and other counter ions through the ion selective permeable membrane and the bulk electrolyte of both cathode and anode chambers (Rozendal et al., 2008). Other factors such as poor electrical contact (especially at the electrodes), electrical conductivity of the electrolyte and high resistivity of the ion selective permeable

membrane could also considerably contribute to ohmic losses observed in MFC systems. The effect of Ohmic losses on MFC performance can be minimised by reducing electrode spacing, use of a high quality membrane and increasing both anodic and cathodic electrolyte conductivities (Hawkes et al., 2010).

Activation losses or activation polarisation are generally related to activation energies expended to initiate anodic and cathodic redox reactions during electron transfer at the electrode/bulk electrolyte interface (Logan et al., 2006). Activation losses could be directly related to the compounds undergoing oxidation in the anode. This compound could be a soluble electron shuttling molecule, cell surface protein or enzyme present at the surface of the bacterial cell or an electron acceptor or catalyst at the cathode. Activation losses are inherent in all chemical reactions including electrochemical reactions. Activation losses in MFCs can be mitigated by improving electrode catalysis and increasing electrode surface area and raising the operating temperature to a maximum level tolerable to the microorganisms.

Bacteria metabolic losses result from the diversion of energy gained by microorganisms (through substrate oxidation) for cell growth and maintenance, instead of being captured by MFC for electricity and voltage generation. The higher the difference in redox potential between the substrate (electron donor) and the anode (temporary electron acceptor), the more energy gain by the microorganism. The high redox potential difference between the substrate and the anode may leads to small potential difference across the terminals of a MFC system (i.e. lower MFC voltage outputs). In order to maximise MFC voltage outputs, the anode potential should be low enough to minimize metabolic losses but not too low to prevent bacteria from switching to fermentative metabolism or other alternative electron

acceptors present in the anolyte (such as sulphates, nitrates and metallic oxides) instead of using the anode as the preferred temporary terminal electron acceptor (Logan et al., 2006).

Concentration losses (or concentration polarisation) occur mainly due to mass transport limitations of chemical species (either oxidising or reducing species) to or from the electrodes especially at high current densities and due to diffusional concentration gradients. At the anode, limited discharge of oxidising species from the surface of the electrode or limited flux of reduced species to the electrode leads to higher oxidising to reducing species ratio at the electrode surface. This results in an to increase in the anode potential which may in turn affect MFC voltage outputs. The opposite of this may occur at the cathode thus leading to decrease in cathodic potential (Logan, 2008). Mass transport limitation in bulk electrolyte can also limit the chemical species flux to the electrode especially in poorly mixed systems. Therefore, concentration losses can be effectively minimised by ensuring adequate mixing of the bulk electrolyte in MFC systems.

Energy losses in MFCs are graphically depicted in polarisation plots as shown in Figure 1.16. In electrochemical cells, system performance and electrochemical characterisations are routinely assessed by polarisation plots and other important parameters such as power density, coulombic efficiency, energy efficiency, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

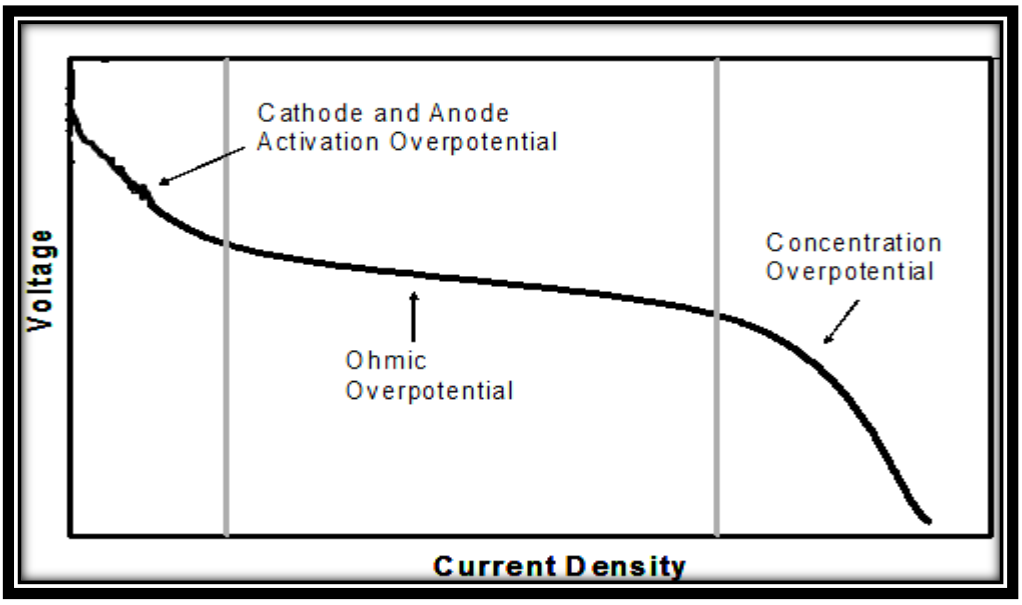


Figure 1.16: Regions of a polarisation curve used to assess the MFC performance depicting the energy losses (Image adapted from http://www.intechopen.com/source/html/16985/media/image17.png)

1.4.5 MFC designs

With over two decades of intensive MFC related research, there have been significant improvements in the architecture and quality of materials used in MFC reactor design and construction to overcome factors limiting the performance of MFCs. There are various kinds of MFC designs ranging from two-chamber, single-chamber to multi-chamber (or stack) systems depending on their applications.

1.4.5.1 Two-chamber MFC systems

The two-chamber MFC design is one of the most commonly used system for fundamental laboratory studies. The two chambered system is a traditional H-type configuration that consists of anode and cathode chambers separated by a separator (e.g. ion selective permeable membrane or salt bridge). These chambers are made up of electrodes housed in glass or plastic materials (Logan et al., 2005). There are different modifications in the designs of two chamber systems (Figure 1.17) made in order to increase electrode surface area, volumetric density and reduced electrode

distance between the two chambers and electrode-based losses (e.g. cylindrical, rectangular, cubical and up-flow tubular MFC systems). Most of the two-chamber systems require the catholyte to be constantly aerated to maintain the availability of atmospheric oxygen for cathodic oxygen reduction reactions. Sometimes ferricyanide (Rabaey et al., 2004) or other oxidising agents/terminal electron acceptors such as hydrogen peroxide, potassium persulfate can be used as catholyte in lieu of oxygen without the need for aeration which requires energy input (Li et al., 2009; Tartakovsky and Guiot, 2006). However, the uses of these alternative electron acceptors in the cathode are not sustainable in terms of costs and long-period availability. The two-chamber systems are not suitable for pilot or large scale applications due to inherent limitations such as low power generation resulting from high overpotential or relatively high internal resistance and design bottlenecks encountered in real applications (Logan et al., 2006).

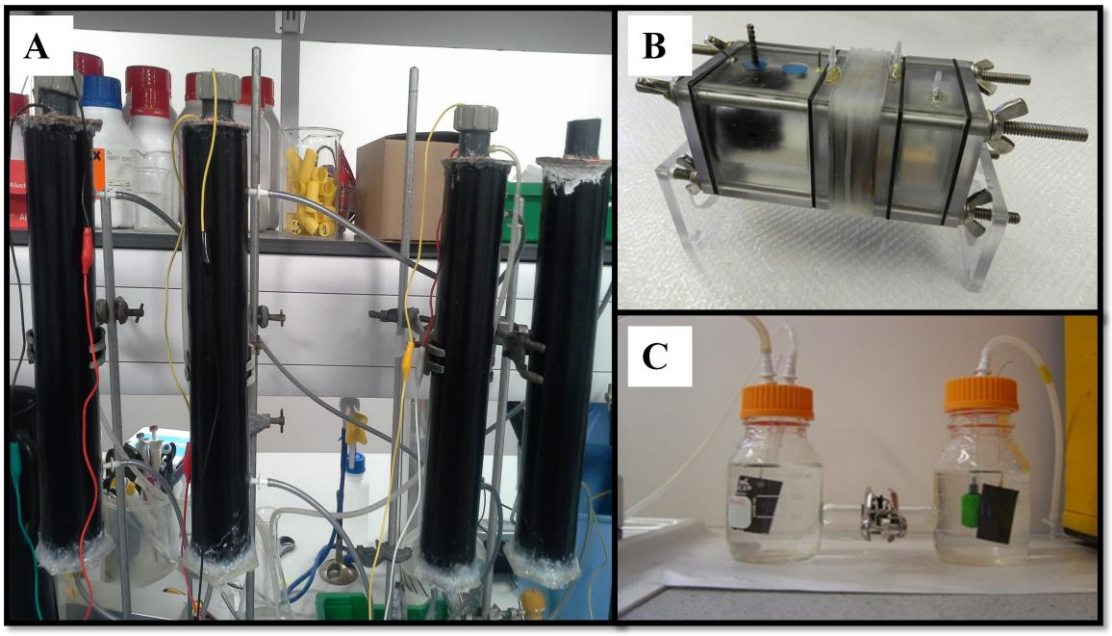


Figure 1.17: Types of dual chambered microbial fuel cells: (A) tubular up-flow type double chamber MFC systems with inner cathode for continuous flow operation (used in this study) (B) the rectangular type and (C) the conventional H-type two-chamber systems with large membrane surface areas (Logan et al., 2006).

1.4.5.2 Single chamber MFC systems

Scaling-up two chamber systems for pilot and industrial applications can be very challenging due to their complex designs and cost as previously described in section 1.4.5.1. Therefore single chamber MFCs were developed to circumvent these design challenges and improve power generation. Single-chamber MFCs consist only of an anode chamber with a porous cathode exposed to air (Logan et al., 2006). The anode is enclosed within the single reactor chamber containing the anolyte medium. Examples of different designs of single chamber systems which can be operated in batch or continuous mode are shown in Figure 1.18.

The single chamber type MFCs are more energy efficient and cost effective in term of their construction compared to their two-chamber counterparts for numerous reasons. In single chamber MFC systems, there is a high degree of flexibility and versatility in reactor configurations to suit intended applications. The distance between the electrodes are highly reduced in single-chamber MFCs compared to traditional two-chamber MFC systems. There is also no need for energy input (usually required for catholyte aeration in two chambers MFC systems) as it uses an air-breathing cathodic system (Hawkes et al., 2010; Logan, 2008). Hence, single-chamber air cathode type MFCs routinely give higher power performance, are more sustainable compared to their two-chamber MFCs and can be fitted for use in continuous MFC operations (Fernando et al., 2013).

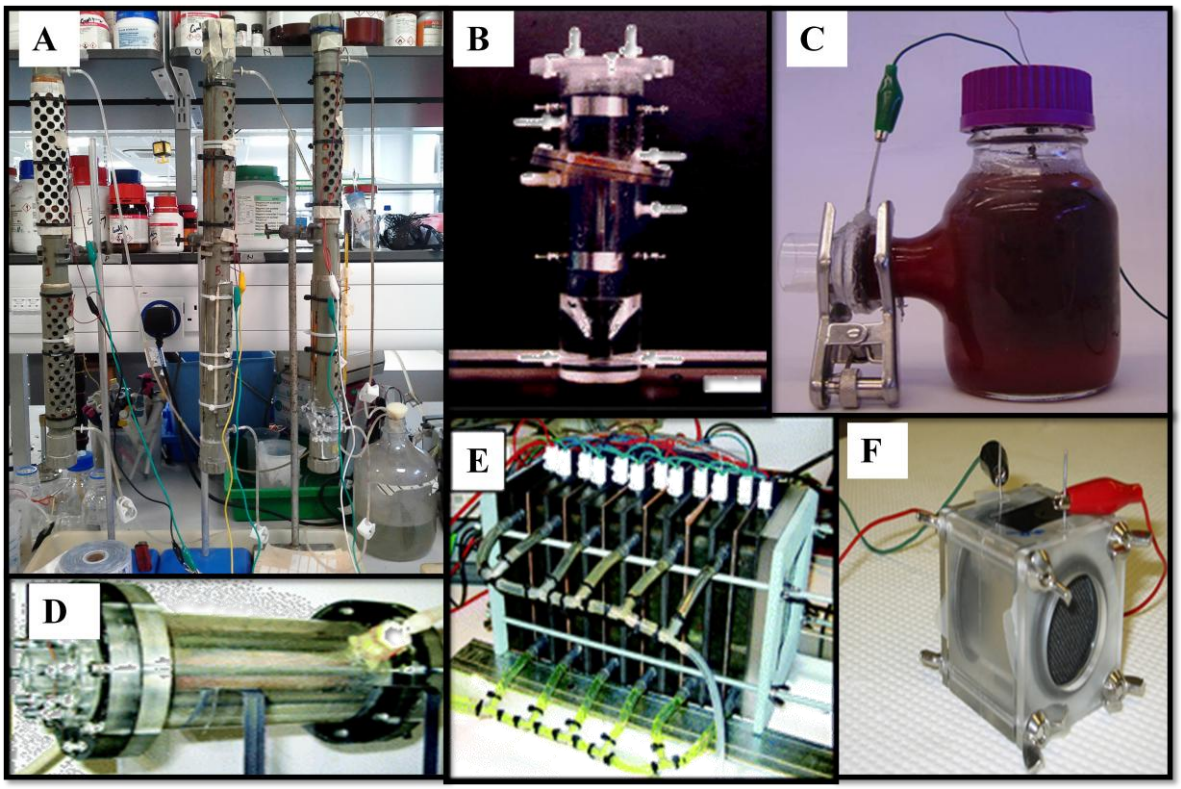


Figure 1.18: Examples of single chamber MFC systems. (A) A tubular up-flow type single chamber MFC for continuous flow operation (used in this study) (B) An up-flow, tubular type MFC with anode below and cathode above, the membrane is inclined (Logan et al., 2006) (C) A single chamber MFC with a very large brush anode and a flat cathode. (D) A single chamber MFC with an inner concentric type cathode for continuous flow operation (Logan et al, 2006). (E) Stacked MFC, with 6 separate MFCs linked together to form a single reactor block (F) Rectangular type single chamber systems for batch operation (Logan et al, 2006).

Sediment MFCs are another kind of MFC design where biogenic electricity is harvested by embedding one electrode (anode) into the marine sediment which is connected through an external circuit to the cathode in the overlying oxic water (Guzman et al., 2010; Donovan et al., 2011).

In sediment MFCs, there is no separator (i.e. ion selective membrane) as the protons released due to substrate oxidation by electrochemically active microbial population present in the sediments are conducted by the seawater via the sediment-water interface (Figure 1.19). This system can be employed in the bioremediation of contaminated sediments and to power some marine electronic devices utilised for

freshwater or marine studies (Donovan et al., 2008). They can also be a reliable source of power for environmental monitoring devices, remote marine devices and serve as unattended systems (unlike conventional batteries) for long periods of operation. The main limitations of sediment type MFCs are low power and voltage generation (Donovan et al., 2011).

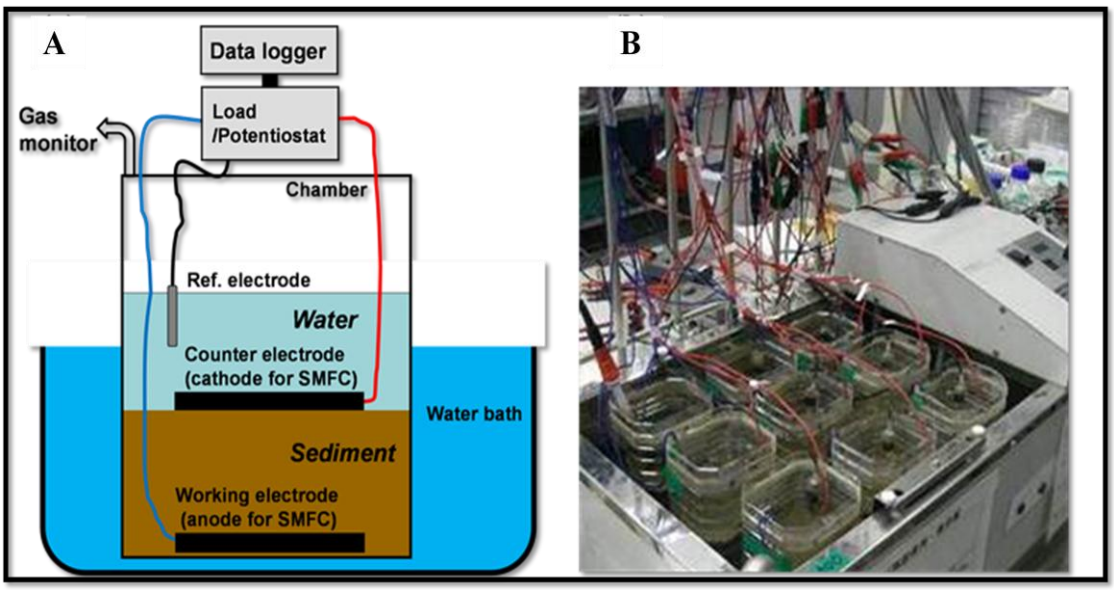


Figure 1.19: (A) Schematic diagram of a sediment-type MFC and (B) the laboratory- based sediment MFC set-up (Ueno and Kitajima, 2014).

Other variations of MFC configuration include miniaturised reactors which are used for remote sensing applications and as bio-screening of environmental strains (Ringeisen et al., 2006). Miniature MFCs also have some limitations such as low power densities and high fabrication costs (Qian et al., 2011).

1.4.5.3 Multi-chamber/stacked MFC systems

In real industrial applications of MFC systems, they must be connected in series or in parallel as a stacked system for significant increase in power generation and overall system voltage outputs (Figure 1.18E). By stacking an appropriate number of individual MFC units together in series or parallel, desired current or voltage can be obtained respectively (Aelterman et al., 2006). However, one of the major challenges

often encountered in stacked MFC systems is voltage reversal which could be due to the loss of microbial activity resulting from fuel/substrate starvation and/or increase in internal resistance at high current density when low external resistance is applied. Voltage reversal can be effectively minimised by connecting the stacked MFCs with a capacitor or by use of high external resistors in individual units (Zhang and Angelidaki, 2012).

1.5 BES applications in bioremediation

BES is an emerging technology that utilises readily oxidisable organic compounds present in wastewater streams for the generation of biogenic electricity or sometimes cathodic hydrogen gas production. BESs are unique in the sense that the microorganisms are able to transfer electrons extracellularly to a solid material like an anode electrode compared to intracellular mechanisms of microbial metabolism observed in non-BESs (e.g. aerobic and anaerobic oxidation of organic substrate in digestor or bioreactors). In addition to energy recovery from wastewater as electricity, other advantages of BESs over conventional wastewater technologies include lower operational costs compared to aerobic treatment (particularly activated sludge system), production of less sludge (Logan and Regan, 2006), bioproduction of high-value chemicals such as ethanol, methane, PHA (polyhydroxyalkanoates) (Steinbusch et al., 2010; Wagner et al., 2009), and capability of operating in psychrophilic and oligotrophic/copiotrophic conditions (compared to anaerobic digestion technology) (Pham et al., 2006).

In the past few years of active research on BES technology, BESs have been used in the treatment of organic and inorganic-containing wastewater from a variety of domestic and industrial sources which include sewage/municipal wastewater (Liu

and Logan, 2004; You et al., 2006a), chocolate industry wastewater (Patil et al., 2009), swine wastewater (Min et al., 2005) and brewery wastewater (Feng et al., 2008) with high COD reductions in the range of 85-95 % and inorganic pollutants such as sulphates (Zhao et al., 2008) and nitrates (Virdis et al., 2008). In numerous previous studies, different types of MFCs operated in either batch or continuous modes at different operating and environmental conditions have demonstrated the ability of MFC systems to effectively degrade or detoxify recalcitrant environmental pollutants found in wastewater with energy recovery.

Due to promising results recorded in the treatment of wastewater and industrial effluents, BESs have been extended to the bioremediation of various types of contaminants, ranging from aromatic or substituted organics (Huang et al., 2011; Wang et al., 2012b; Zhang et al., 2010b) to heavy metals (Gregory and Lovley, 2005; Huang et al., 2010; Abourached et al., 2014) and inorganic pollutants such as nitrates and phosphate compounds (Tong and He, 2013; Ichihashi and Hirooka, 2012; Zhang et al., 2012a) present in contaminated soil and groundwater systems.

BESs for bioremediation are a promising application but being a young and advancing field of research, only a few studies have been undertaken. Some of the recent studies highlighting BES systems used for pollutant removal (especially petroleum hydrocarbons) are summarised in Table 1.1.

Table 1.1: A summary of studies that utilised BES for the purpose of removal of recalcitrant pollutants.

Recalcitrant pollutant	**BES configuration & Electron donor / acceptor**	**Micro-organisms**	**Operating mode**	**Removal efficiency / rate[a]**	**Reference**
Nitrobenzene	Two-chamber MFC, acetate/nitrobenzene	Mixed culture in the anode	Continuous	1.29	Mu et al., 2009
Furfural	Single chamber MFC, Furfural/ferricyanide and oxygen	Adapted mixed culture	Batch	95 %	Luo et al., 2010
1,2-dichloroethane	Two-chamber MFC, 1,2-dichloroethane /ferricyanide and oxygen	Microbial anaerobic consortium	Continuous	95 %	Pham et al., 2009
4-nitrophenol	Two-chamber MFC, Glucose/4-nitrophenol with H_2O_2	Anaerobic sludge	Batch	100 %	Zhu and Ni, 2009
Petroleum hydrocarbons	U-tube single chamber MFC, petroleum hydro-carbons/oxygen	Mixed microbial flora from petroleum-contaminated saline soil	Batch	15.2 %	Wang et al., 2012b
2-chlorophenol	Two-chamber MEC, acetate /2-chlorophenol	*Anaeromyxobacter Dehalogenans* [b]	Batch	86 % / 0.04	Strycharz et al., 2010
Perchlorate	Two-chamber MFC, acetate/perchlorate	Denitrifying microbial community [b]	Batch	97 %	Bulter et.al., 2010
Pyridine	Two-chamber MFC, Glucose/Pyridine	Pre-acclimated mixed microbial culture	Batch	95 %	Zhang et.al., 2009

Table 1.1 cont'd

Recalcitrant pollutant	BES configuration & Electron donor / acceptor	Micro-organisms	Operating mode	Removal efficiency / rate[a]	Reference
Quinoline	Two-chamber MFC, Quinoline/ferricyanide	Mixed culture	Batch	99 % / 4.1	Zhang et.al., 2010b
Trichloroethene	Two-chamber MEC, H_2 (g) /Trichloroethene	Mixed culture	Batch	0.001	Aulenta et al., 2007
Coal Tar in wastewater	Two-chamber tubular MFC, coal tar /oxygen	Activated sludge from coal tar refinery	Batch	88 %	Park et al., 2012
Toluene	Two-chamber MFC, Toluene /ferricyanide	Anaerobic sludge	Batch	100 %	Lin et al., 2014
Phenanthrene, Pyrene	Sediment MFC, phenanthrene, pyrene /overlying oxic water	Mixed microbial community from PAH-contaminated sediment	Batch	Phenanth-rene :99 % Pyrene: 95 %	Yan et al., 2012
Gold	Cubical two-chamber MFC, Acetate / Gold	Microbial anaerobic consortium	Anode: Continuous Cathode: Batch	99 %	Choi and Hu, 2013
Nitrate	Two-chamber MFC, Acetate / Nitrate	Adapted mixed microbial community	Batch	90 %	Zhang and He, 2012

[a] Calculated on the basis of net cathodic compartment ($molm^{-3}\ d^{-1}$).
[b] at the cathode

In addition to the selected studies on removal of petroleum hydrocarbons as listed above, Morris et al (2009) achieved 82 % DRO (diesel range organics) removal with an MFC over 21 days compared to an anaerobic incubated control, which achieved 31 % removal. Morris and co-workers' work is a typical example of an *ex situ* treatment of diesel-contaminated groundwater collected from a nearby refinery. By applying a U-tube soil MFC, a model *in situ* treatment process, Wang et al (2012b) were able to enhance the degradation of petroleum hydrocarbons (collected from oil-contaminated sediment) close to the anode (<1 cm) by 120 % within 25 days in a lab-based soil MFC compared to non-MFC conditions. Thus there is evidence to suggest that MFCs could be used for both *in situ* and *ex situ* bioremediation although only a few studies have been reported. BESs have recently attracted much attention owing to their eco-friendliness, robustness, biocompatibility, versatility and production of useful products (e.g. electricity, fuels and chemicals).

1.6 Other applications of BES technologies

1.6.1 Microbial electrosynthesis:Bioproduction of alternative chemical products

Microbial electrosynthesis (MES) is a bioelectrochemical technique that involves microbially catalyzed synthesis of chemical compounds in an electrochemical cell which is mostly associated with the reduction of carbon (IV) oxide, CO_2. Microbial electrosynthesis can be applied for production of fuels and chemicals such as the production of ethanol, methane, H_2O_2, caustic soda and PHAs (Rabaey et al., 2010; Rabaey and Rozendal, 2010; Steinbusch et al., 2010; Wagner et al.,2009; Srikanth et al., 2012). Few laboratory-scale studies have recently demonstrated the use of acetogens and other cathodic-electrogenic microbes that have the ability to convert various syngas components (CO, CO_2, and H_2) to multi-carbon compounds, such as acetate, butyrate, butanol, lactate and ethanol (Srikanth et al., 2012). In MES,

methane and other carbon-based compounds production can be coupled to CO_2 capture, this may offer good perspectives for industry to reduce their greenhouse gas emissions and does not compete with food chain production (Steinbusch et al., 2008). However, this promising BES technology is still in its infancy and active research is ongoing.

1.6.2 As a biosensor

BES application have been extended to biosensors where the biocatalyst in the anode chamber acts as a biological detector (as a response to a wide range of environmental stimuli) to evaluate the performance of the system (Kaur et al., 2013). The electric current generated from the BES is directly proportional to the concentration of available organic contaminants. This microbial device can be used as a biochemical oxygen demand (BOD) sensor in assessing microbial activity in the environment especially in subsurface environments (Kim et al., 2003; Zhou et al., 2012). BES biosensors can also be used as a pollutant biomonitoring system for real-time detection of the release of toxic substances and specific molecules into or in water sources/bodies (Shen et al., 2012). Micro-MFC has recently been developed for high-throughput screening and sensitivity analyses of biological and electrochemical performance parameters (Alister et al., 2012). Similarly, BES biosensor system could be readily expanded for monitoring of toxicity, endocrine levels, detection and bioavailability of specific compounds in bioremediation, pharmaceutical, food and other allied industries.

1.6.3 Electrowinning

Electrowinning is the electrodeposition of metals of a leached solution from their ores. In recent times, BES have been used in the electrowinning of valuable metals coupled with wastewater treatment and sometimes, bioelectricity generation (Choi

and Hu, 2013; Wang and Ren, 2014). Precious metals can be recovered effectively using MFC technology where the redox potential of the metals are higher than the anode potential. At high redox potential relative to the anode, redox process is thermodynamically favourable, hence the generation of electricity with metal recovery at the cathode. Examples of such metals include gold, copper, silver, chromium and selenium (Choi and Cui, 2012; Heijne et al., 2010; Lefebvre et al., 2012a; Tao et al., 2012; Wang et al., 2011; Zhang et al., 2012b).

However, at low cathodic redox potential relative to the anode, the redox reaction is not spontaneous and thus requires a little energy input (in form of externally applied volatage) to forcibly drive the flow of electrons to the BES cathode for metal cathodic reduction. MEC systems are employed in the recovery of such metals with low redox potentials which include nickel, lead, zinc and cadmium (Modin et al., 2012; Qin et al., 2012). The BES platform could be an alternative, sustainable and cost effective technology for the effective reduction and recovery of different metals from metallurgical wastewater and other industrial sources with no or limited energy input.

1.7 Current challenges in BES applications

Being a new technology, BES are still faced with many challenges e.g. low microbial growth rate, electron diversion due to competition amongst various electron acceptors (e.g. nitrates and sulphates), overpotential losses at electrodes, and high cost of materials e.g. the use of platinum as a catalyst at the cathode. In addition, acidification of the anode due to pH gradients resulting from membrane under-performance among others things, greatly tests BES wide-applicability (Franks and Nevin, 2010; Rosenbaum and Franks, 2014). For instance, use of phosphate buffer in

the anode aids in maintaining neutral pH but only for short periods. As the ionic strength of the buffer depletes, protons build up in the anode resulting into anode acidification, which may inhibit microbial activity or causes overpotential at the anode (Pant et al., 2010). Other challenges encountered in BESs are substrate non-bioavailability (e.g. insoluble PAHs), bottlenecks in scale-up operations, ohmic losses and a lack of robust understanding of the microbiology of microbes with regards to electron transfer. Limited knowledge exists on the mechanism of extracellular transfer of electrons to the electrode's surface although there are suggestions that redox mediators may aid electron transfer processes (Rosenbaum and Franks, 2014; Clauwaert et al., 2008).

In previous studies, microcosms were employed in the bio-treatment of PAHs in soil and wastewaters (Morris and Jin, 2012; Wang et al., 2012b). To the best of my knowledge, very few reports exist on the use of electrochemically active microorganisms - either in pure or defined mixed cultures in enhancing biodegradation rates (particularly petroleum hydrocarbons) in MFCs. Thus bioaugmentation, a widely used strategy in traditional bioremediation (Okoro, 2010), has not been fully exploited in BESs especially for petroleum hydrocarbons like PAHs. Since not all microorganisms can naturally produce their own electron shuttles, addition of redox mediators could enhance electron transfer rates. Bioavailability is a key issue with petroleum hydrocarbons and could be influenced by the use of synthetic non-ionic surfactants e.g. Tween 80 or use of microorganisms (e.g. *Pseudomonas aeruginosa*) that produce rhamnolipids (which are biosurfactants). Factors influencing the performance of MFCs such as type of inocula (axenic or mixed cultures), nature and concentration of substrate and

environmental factors (such as temperature and salinity) could potentially influence the bioremediation process.

Also due to the high cost of platinum which is used in making chemically-catalyzed cathodes, possible replacement with other electrocatalysts with similar performance or the use of biocathodes would alleviate significantly the high cost of BES reactor design in large-scale field applications. All parameters mentioned above may be applicable to both *ex situ* and *in situ* treatments. Due to high sorption of PAHs to surfaces, PAHs are mostly found in soils and sediments. It is possible that soil MFC could be used to remediate PAHs in soil microcosms, given the right conditions e.g. moisture content and redox potential.

1.8 Aim and Objectives of this research

The overarching aim of this research project was to develop a laboratory-based MFC system for the degradation of phenanthrene and benzene coupled with concomitant electricity generation that is effective, efficient, at different treatment conditions and applicable to liquid and particulate systems.

The hypothesis for this research was that MFCs could enhance the treatment of selected petroleum hydrocarbons (i.e. phenanthrene and benzene) in groundwater and soil microcosms coupled with concomitant energy recovery. The specific objectives to be met in order to fulfil the above overall aim included the following:

1.8.1 Specific Objectives

1. **To investigate the degradation of model BTEX & PAH compound (i.e. benzene and phenanthrene respectively) in the anode of dual-chamber MFCs.**

Biodegradation of phenanthrene and benzene were investigated individually in dual-chambered microbial fuel cells (MFCs) using a range of inoculum type (*Shewanella oneidensis* MR1 14063, *Pseudomonas aeruginosa* NCTC 10662, mixed cultures and their combinations thereof)(section 2.6.1). Best performing strains were selected based on the assessment of MFC performance (Chapter 3).

2. **To investigate the influence of different treatment conditions on degradation of petroleum hydrocarbons mixtures using hydrocarbon-acclimated mixed microbial consortia in MFCs.**

   Microbial fuel cells need to be robust if they are to be applied in the field for bioremediation. In order to assess the robustness of the MFC system, the performance of MFCs fed with a mixture of benzene and phenanthrene at various temperatures and salinities; and supplemented with different mediator types using adapted anaerobic consortia was investigated. Other key factors affecting MFC performance such as substrate concentration, external resistance, surfactant type and choice of cathodic electron acceptors were also investigated. Microbial community dynamics during MFC operation was investigated using 16s rDNA microbial community profiling methods (Chapter 4).

3. **To investigate the interaction of critical factors (such as redox mediator, salinity and external resistance) in an aqueous MFC for the degradation of phenanthrene and benzene mixtures using response surface methodology (RSM).**

   In a previous study, overall MFC performance for removal of petroleum hydrocarbons mixtures at different treatment conditions individually was investigated (as described in chapter 4). However, in field applications, one or

more influencing factors (interacting with one another) that could affect system performance are often encountered (Mohajeri et al., 2010). Conventional methods of studying a process by keeping other factors constant does not depict the combined effects of all the factors involved. In order to evaluate the interactive effects of critical factors and optimise MFC performance, a well-known statistical tool, response surface methodology (RSM) was employed. Parameters of significant interest selected were salinity, external resistance and redox mediator (Chapter 4).

4. **To investigate the degradation of phenanthrene and benzene in a continuous operation using two different tubular MFC designs which could be suitable for both *in situ* and *ex situ* applications.**

   BES systems need to be uniquely designed (in terms of MFC architecture) and tested for their performance before their possible deployment for either *in situ* or *ex situ* applications. The two BES systems used in this study were operated in a continuous mode to test their long term operational stability and robustness at ambient temperatures. A petroleum hydrocarbon-acclimated mixed microbial culture was used in these MFC systems to obtain optimum degradation of the petroleum hydrocarbon mixture with energy recovery. Other important aspects of operation such as the hydraulic retention time (HRT), toxicity assessment of degradation products and the reactor's response to low and high nutrient conditions were also investigated (Chapter 5).

5. **To design and investigate the performance of a lab-scale MFC (a tubular design) for enhanced bioremediation of phenanthrene-contaminated soil.**

The last part of this project focused on designing a bioelectrochemical system for the remediation of phenanthrene-contaminated soil that could be deployed directly as a prototype-MFC design in field applications. Due to the bioavailability problems associated with phenanthrene in soil matrix, the effect of surfactant addition on MFC performance was investigated (Chapter 6).

1.9 Organisation of the Thesis

This thesis has 10 chapters with chapter 3-6 showing the results and discussion (which underpins the specific objectives) accompanied by concluding remarks about key findings. A list of references is provided at the end of the thesis. Also, some additional information, which may be important to the reader, but not included in each chapters are provided in the appendices section before the list of references. The thesis overview is as follows:

- Chapter 1 provides an introduction and the rationale for the research. It also gives a comprehensive review on petroleum hydrocarbons, their degradation in the environment, existing conventional remediation technologies and BES systems. Based on the reviews, research opportunities are identified and research objectives formulated.
- Chapter 2 provides information on experimental methods and procedures including MFC designs & operation and analytical procedures employed to carry out the research work.
- Chapter 3 reports the results and discussion on biodegradation of phenanthrene and benzene in dual-chambered microbial fuel cells (MFCs) using a range of inoculum types.
- Chapter 4 reports the results and discussion on the robustness of MFCs at different treatment conditions with petroleum hydrocarbon-synthetic

wastewater as fuel. This chapter also provides the results and discussion on the interactive effect of critical operating factors (such as redox mediator, salinity and external resistance) on the performance of MFC fed with petroleum hydrocarbon.

- Chapter 5 reports results and discussion on the application of BES systems to the treatment of synthetic groundwater and refinery wastewater using scaled-up tubular MFCs.
- Chapter 6 provides results and discussion on the enhanced anaerobic degradation of phenanthrene in contaminated soil using a lab-scale soil MFC system.
- Chapter 7 gives a summary of all the results in the research work.
- Chapter 8 highlights potential areas for future work in this research area.

CHAPTER 2:

EXPERIMENTAL METHODS

2 Experimental methods

2.1 Chemicals and reagents

Chemicals and reagents were obtained from Sigma Aldrich (Dorset, UK), VWR (UK), Acros (UK) and QiaGen Ltd (Crawley, UK). Chemical solvents including Ficodox Plus™ mixed COD reagent were obtained from Fisher Scientific (Loughborough, UK). Reagents and enzymes for polymerase chain reaction (PCR) were purchased from New England Biolabs (USA). All molecular biology grade reagents for denaturing gradient gel electrophoresis (DGGE) analysis were purchased from Sigma Aldrich, UK. All chemicals were of analytical grade (≥99 % purity) and used without further purification**.**

2.2 Microorganisms and media compositions

Two pure strains along with an undefined anaerobic consortium were used as anodic biocatalysts. *Shewanella oneidensis* (MR1 14063) and *Pseudomonas aeruginosa* (NCTC 10662) were obtained from microbial culture collections of the University of Westminster, UK. All bacterial strains were maintained as glycerol cryopreserved stock cultures at -80℃ till ready for use. These strains were selected for this study because they are electrochemically active, facultative anaerobic microorganisms that are ubiquitous in the environment. Anaerobic digested sludge samples were obtained from Mogden Sewage Treatment Works, London (UK). The defined minimal medium for bioelectrochemical experiments described in chapters 3-5 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). The compositions of Wolfe trace mineral and vitamin mix solution are given in Table 2.1. Anaerobic sludge inoculum was grown anaerobically in serum vials in minimal medium supplemented with D-

glucose (500 mg L^{-1}) as primary carbon source and subsequently incubated at 30℃ for 48 h.

Table 2.1: Components of the trace elements and vitamin mix stock solution (100X concentrated) used in this study.

Composition of vitamins mix		**Trace elements composition**	
Component	**Concentration (mg L^{-1})**	**Component**	**Concentration (mgL^{-1})**
P-aminobenzoic acid	5	Nitrilotriacetic acid (NTA)	1500
L-ascorbic acid	5	$MnSO_4.H_2O$	500
Folic acid	2	$FeSO_4.7H_2O$	100
Riboflavin	5	$CoCl_2.6H_2O$	100
Nicotinic acid	5	$ZnSO_4.7H_2O$	100
D-calcium pantothenate	5	$CuSO4.5H_2O$	10
Thiamine hydrochloride	5	$AlK(SO_4)_2.12H_2O$	10
Biotin	2	H_3BO_3	10
Pyridoxine hydrochloride	10	$Na_2MoO_4.2H_2O$	10
Thioctic acid	5	$NiCl_2$	10
Vitamin B12	0.1	$NaWO_4.2H_2O$	5

S. oneidensis was grown in minimal medium supplemented with sodium pyruvate (500 mg L^{-1}) and 500 mg L^{-1} casein hydrolysate (Sigma Aldrich, UK) as carbon source while *P.aeruginosa* was sub-cultured in minimal medium supplemented with 300 mg L^{-1} D-glucose. *Vibrio fischeri* (13938) used for bioluminescence toxicity assays was purchased from NCIMB (UK) and was grown in oceanibulbus growth medium (NCIMB growth media catalogue) (Table 2.2). All media preparations were

autoclaved at 121℃ for 15 min, except for vitamins, mineral and sucrose solutions that were filter-sterilized (0.2 µm) (Millipore, UK).

Table 2.2: Composition of Oceanibulbus medium for *V.fischeri* (13938)

Component	**Concentration (g L^{-1})**
Tryptone	10
Yeast extract	5
NaCl	10
Sigma Aldrich sea salts ready mixture (S9983)	14

2.3 Acclimation of anaerobic sludge for MFC studies

Anaerobically digested sludge previously obtained from Mogden Sewage Treatment Works London (UK) was initially acclimated at 30℃ for six months in a Winogradsky column (35 cm long, 5 cm wide) containing all BTEX- benzene, toluene, ethyl benzene and xylene (each of the BTEX components at 100 mg L^{-1}) and four PAHs compounds which comprises of pyrene, naphthalene, fluorene and phenanthrene (each at 100 mg L^{-1}) supplemented with microcrystalline cellulose (1 % w/v) as carbon source, calcium carbonate (0.25 % w/v) and calcium sulphate (0.5 % w/v). This was done in order to enrich petroleum hydrocarbon degrading bacteria for subsequent experiments. The petroleum hydrocarbons mixture was replenished in the Winogradsky columns (by adding 100 mg L^{-1} of each of the components of the petroleum hydrocarbons) twice a month in order to maintain substrate availability and microbial viability. Acclimatized anaerobic sludge samples were taken from the lowest level (bottom layer) of the Winogradsky column (in the region dominated by anaerobic microorganisms) and the inoculum was subsequently grown anaerobically

in serum vials in minimal medium supplemented with D-glucose (Sigma Aldrich, UK) and incubated at 30℃ for 48 h in an anaerobic jar before subsequent use in MFCs. This adapted mixed culture (or adapted microbial consortium) obtained from the Winogradsky column after six month acclimation was used in studies described in chapters 3,4,5 and 6.

2.4 Soil sample collection and characterization

Contaminated soil samples used for soil MFC studies were obtained near Barking, London, UK. The sampling location has a known history of diesel and engine oil contamination. The soil sample was taken from 5 to 10 cm below ground surface. They were stored in hermetically sealed plastic bags, transported directly to the laboratory , air dried for three days at room temperature (23 ± 3℃) and finally passed through a 2-mm sieve to remove plant roots and rocks. The sieved soil samples were stored at 4℃ before use. A complete physicochemical analysis 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 mg kg^{-1} DS (dry soil). The main characteristics of the soil samples are described in Appendix 1. This soil was used in MFCs for the work presented in chapter 6.

2.5 Reactor designs, configurations and operations.

2.5.1 Reactor configuration and operation for H-type dual chambered MFCs.

For the work described in chapters 3 and 4, the bioelectrochemical reactor (Figure 2.1) was constructed by assembling two identical Plexiglass bottles with inner diameter of 7 cm (total volume of 250 mL, working volume of 200 mL) separated by a cationic exchange membrane (20 cm^2, CMI-7000 Membrane International NJ, USA). The glass bottles were held firmly together by a metallic clip with two rubber gaskets placed in-between to ensure the reactor was airtight. The electrodes were

made of carbon felt (C-TEX 27; surface density 110 g/m^2; surface area 1100 m^2/g, Mast Carbon Inc, Basingstoke, UK) of the same size (4.0 cm × 5.0 cm) but for the cathode, the carbon felt was coated with a platinum (Pt) catalyst (0.50 mg/cm^2) on both sides. 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 % (w/w) 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.

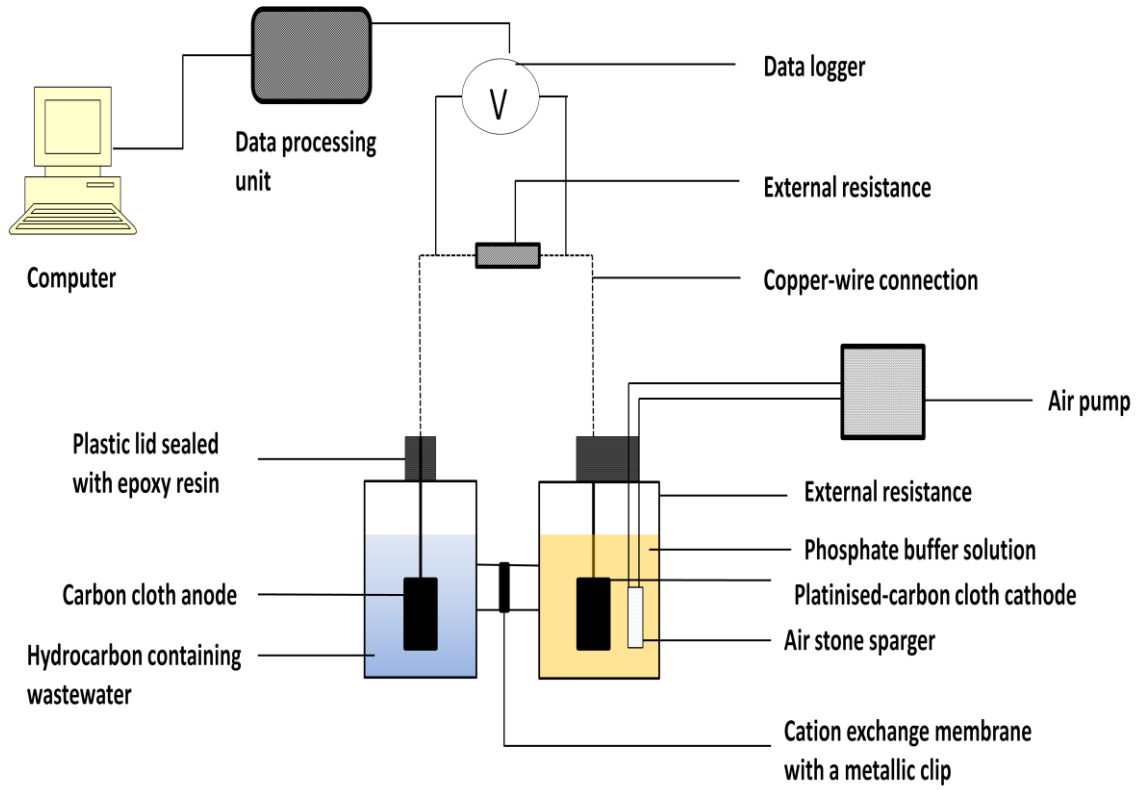


Figure 2.1: Schematic diagram of the H-type dual chambered-MFC set-up.

Electrode connections were made by soldering insulated copper wire onto the electrodes using a soldering iron kit. All exposed connections were coated with non-conductive epoxy resin for insulation. The anode chamber was filled with anolyte

medium (pH 7.0). The catholyte and anolyte solutions were buffered to pH 7.0 using 100 mM phosphate buffer in all experiments. The cathode chamber was continuously sparged with air (at a flow rate of 100 $mLmin^{-1}$) using an aquarium pump (KOI Air, UK).

The MFCs were sterilized by autoclaving at 121℃ for 15 mins followed by addition of anolyte to the anode chamber which was done aseptically. All experiments were operated in semi-batch mode. The MFCs were inoculated with 20 mL of actively growing culture at start up only for experiments described in section 2.6.1. For others experiments described in sections 2.6.2 and 2.6.3, MFCs were seeded with inoculum taken periodically from a running, non-experimental MFC. Anaerobic conditions were maintained in the anode chambers by purging them with 100 % nitrogen gas for 15 mins before MFC operation began. The electrodes were connected via an external circuit containing a single external resistor of 1000 Ω in all experiments and voltage outputs logged in real-time using Picolog ADC-24 data acquisition software (Pico-Technology, UK). All experiments were conducted in a temperature regulated Stuart 160D incubator (Fisher Scientific, UK). The MFCs were operated at 30℃ for all experiments unless otherwise stated.

2.5.2 Reactor configuration and operation for tubular MFCs

Two different designs of tubular MFCs were used in this study. The first design (Figure 2.2) for a possible *in situ* application 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) made up the cathode chamber with working volume of 300 mL. The anode chamber (total working volume of 500 mL) comprised of the outer concentric PVC pipe with inner diameter,

6.5 cm and length, 35 cm. Both the anode and 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 throughout experiments. All MFCs 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 mg L^{-1} at pH 2) was used as catholyte in the cathode feeding tank. The anode feeding tank was filled with petroleum hydrocarbon containing synthetic wastewater (section 2.6.3). The petroleum hydrocarbon containing wastewater and bromate solution influent feeding rates were varied simultaneously throughout the study. All tubular MFCs were operated continuously at ambient temperature which varied from 14℃ to 23℃ corresponding to winter and summer periods respectively. Notably, all the tubular MFCs (i.e. both MFC designed for *in situ* and *ex situ* applications) 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) per anode volume (500 mL). The running MFC was previously seeded with an inoculum of the adapted mixed culture previously described in section 2.3. 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.

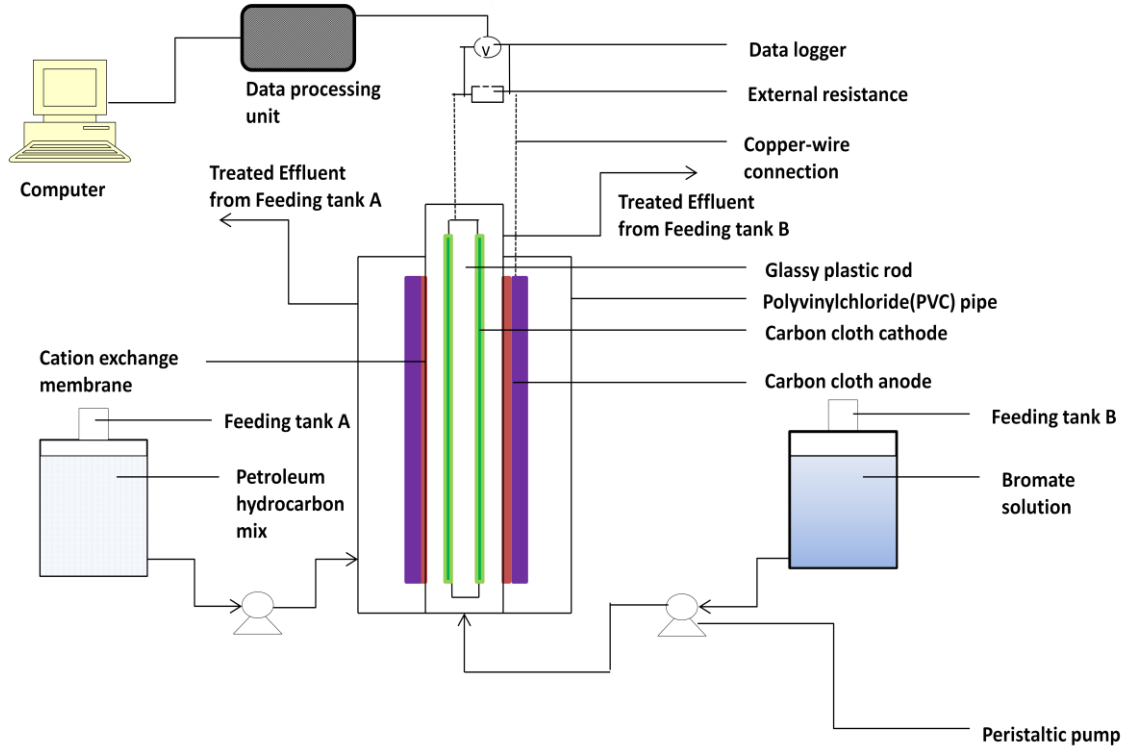


Figure 2.2: A schematic diagram of the tubular MFC reactor in an up-flow configuration for the treatment of hydrocarbon-containing synthetic wastewater for *in situ* applications.

The second MFC designed for possible *ex situ* applications were tubular single chamber MFCs which were constructed from PVC pipes and had a total working volume of 400 mL (Figure 2.3). 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 placed between the anode and the cathode. Electrode spacing was approximately 1.3 cm.

The hollow concentric anode ran through the length of the PVC tube. Pt powder was used as the oxygen reduction catalyst in the cathode and was coated onto the cathode membrane-facing side at Pt/C loading of 0.35 mg/cm^2.The air facing side of the cathode contained a PTFE diffusion layer in order to minimise water loss through the cation exchange membrane. The cathode catalyst layer was applied as described in section 2.5.1 and 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 and the connection interfaces were insulated with non-conductive epoxy sealant. 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 during the experiments using a Picolog ADC-24 (Pico Technology, UK) data logging system. Petroleum hydrocarbon- wastewater was continuously fed in an upward-flow configuration 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 2.6.3.

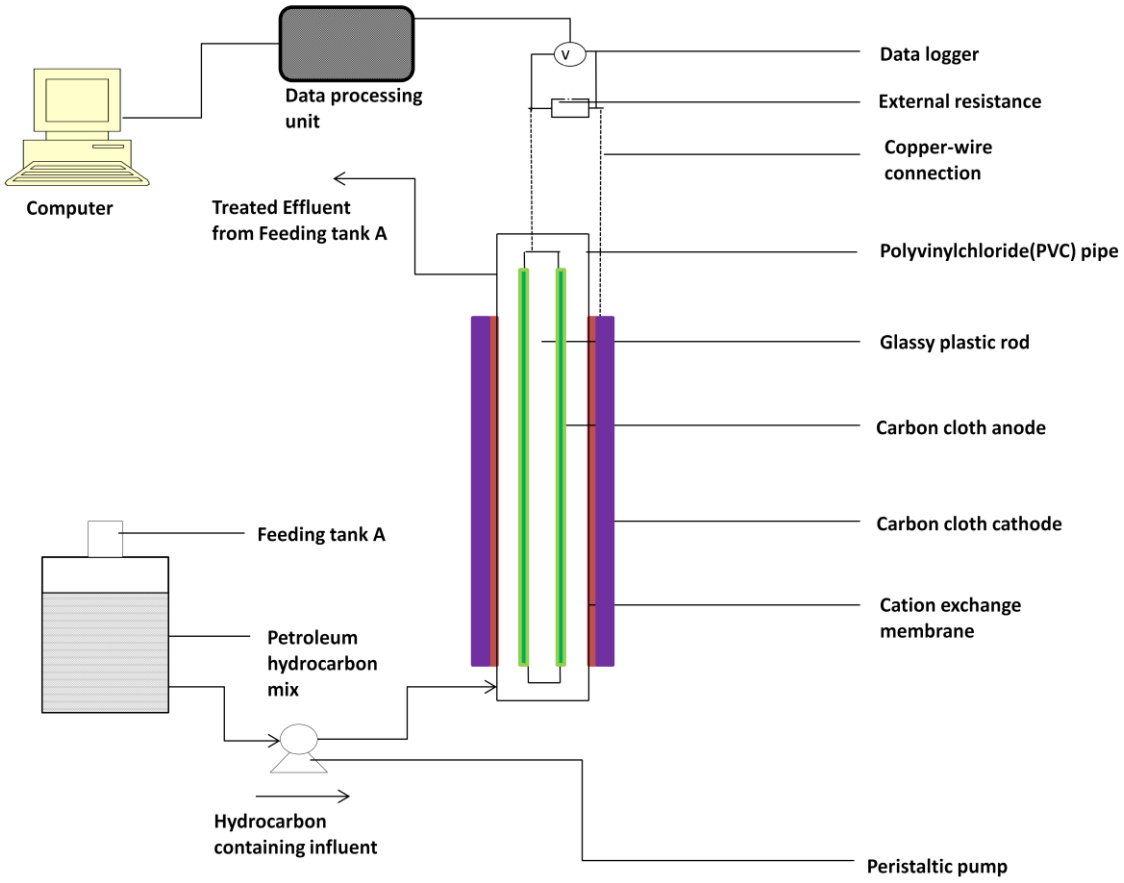


Figure 2.3: Schematic diagram of the tubular MFC reactor in an up-flow configuration used for the treatment of petroleum hydrocarbon containing synthetic wastewater for a possible *ex situ* application.

2.5.3 Reactor design and set-up for a tubular soil MFCs

The soil MFCs are very similar in design to the tubular MFCs described in section 2.5.2. The MFC reactors were constructed using PVC tubes with one sealed end as shown in Figure 2.4. 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 working 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 separated by the 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 at intervals to make up for water loss due to evaporation. The soil MFC was established by inserting the constructed MFCs into 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.

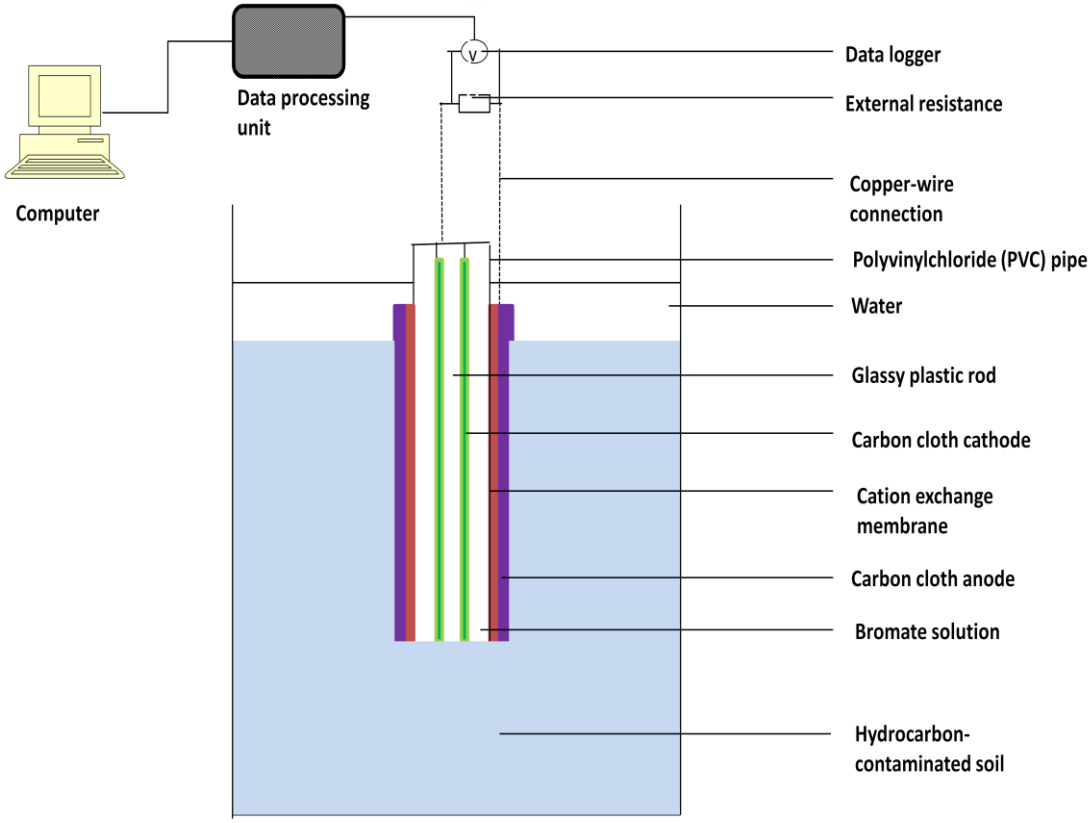


Figure 2.4: The schematic diagram of the tubular soil MFC reactor experimental set up employed for treating a model PAH-contaminated soil.

Both the anode and cathode electrodes were constructed from carbon felt (C-TEX 27; Mast Carbon Inc, Basingstoke, UK) with projected surface area of 156 cm^2 and 96 cm^2 (measured) respectively. Electrical connections on the electrodes were secured by insulated soldering copper wire onto the electrodes and all naked junctions were sealed with non-conductive epoxy to avoid electrical short circuit and electrode

corrosion. The soil MFC was loaded with an external resistance of 1000 Ω and incubated for 60 days at ambient temperature (23 ± 5℃) without exogenous control of temperature.

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

2.6 Experimental design

2.6.1 Degradation studies on petroleum hydrocarbons using different inoculum types (Objective 1)

2.6.1.1 Biodegradation of phenanthrene in MFCs

The influence of inoculum type on phenanthrene degradation and MFC performance was investigated using *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS) and lastly anaerobic digested sludge with *P. aeruginosa*, MCP (Table 2.3). For inoculum containing the mixture of two different culture, they were grown in different growth media individually as described in section 2.2 and the mixture prepared at the ratio of 1:1. Each inoculum was 10 % v/v of the working volume of the anode chamber (200 mL) for all MFC reactors used in these experiments. The measured optical density (OD) of each inoculum used in this study using different strains combinations was 0.6 at 600nm by following a method described in section 2.7.2. The anodic chamber had a total capacity of 250 mL and a

working volume of 200 mL. The anolyte medium consisted of 100 mg organic substrate (pyruvate or glucose) per litre of minimal medium (as earlier described in section 2.2), 20 mg L^{-1} phenanthrene (taken from a 1000-fold concentrate in 100 % methanol) and the inoculum (20 mL). In each treatment, three controls were employed: an abiotic MFC, disconnected MFC and non-MFC (anaerobic control using the same strains as in the test). The abiotic control experiments used in this work were not seeded with the microbial inoculum but used the same medium and were assembled and operated under the same conditions as the tests. The amount of methanol used (about 350 mg L^{-1}) in dissolving the phenanthrene was considered to be non-toxic since the concentration used is far below the minimum inhibitory concentration for microorganisms (Wadhwani et al., 2009; Caldwell, 1989). The experiment was carried out in dual chamber MFCs assembled and operated as described in Section 2.5.1. Exactly 2ml of samples collected from the MFCs at every 12 h interval during MFC operations were analysed for COD removal rates, phenanthrene degradation efficiencies and COD removal efficiencies using methods described in sections 2.7.1 and 2.7.3. Power density measurement and polarisation readings were taken at peak voltage during MFC operation for all experiments (see section 2.7.4). MFC performances of all inocula were assessed based on their degradation performances and electrochemical performances. The system performance index, **K** was calculated as;

$$\mathbf{K} = xyz / 1000 \quad \text{..} (12)$$

where x, y and z represents degradation rate (mg $L^{-1}h^{-1}$), maximum power density (mWm^{-2}) and COD removal (%) respectively. A high **K** value is an indication good system performance.

Table 2.3: Summary of cultures used in each experiment in MFCs for phenanthrene degradation studies.

	Strain used			**Inoculum type**
Experimental run	*P. aeruginosa*	*S. oneidensis*	Undefined mixed culture	
1	-	+	-	S.O*
2	+	-	-	P.A
3	+	+	-	CO (P.A & S.O)
4	-	-	+	MC
5	+	+	+	MCO
6	-	+	+	MCS
7	+	-	+	MCP
8	-	-	-	Abiotic control

*Glucose (100 mg L^{-1}) was used as organic substrate in all experiments except for *S. oneidensis* (S.O) where pyruvate (100 mg L^{-1}) was used in lieu of glucose.

2.6.1.2 Adsorption of phenanthrene onto the anode (carbon electrode)

For work presented in chapter 3, this experiment determined the adsorption equilibrium of phenanthrene between the bulk solution in the anode and the carbon-felt electrode, (C-TEX 27; surface density 110 g/m^2; surface area 1100 m^2/g, Mast Carbon Inc, Basingstoke, UK). Adsorption of hydrocarbons on the anode electrode was expected and could be misinterpreted if only liquid samples from the anode were considered for analysis. Various phenanthrene concentrations - 20, 40, 80, 140 and 200 mg L^{-1} were prepared in conical flasks containing 100 mL minimal medium (similar to anolyte medium) and a carbon electrode (total surface area of 40 cm^2). The pH of the medium was buffered at pH 7 by phosphate buffer solution (PBS, which is part of the medium composition).

The flasks were placed inside an incubator with the agitation speed set to zero, to replicate conditions used in the running of the MFCs (described in section 2.6.1.1). Flasks containing no electrodes were used as controls. All flasks were kept at a constant temperature of 30℃, with a contact time of 180 mins. To clarify the influence of a negative anode potential on phenanthrene adsorption in abiotic MFCs,

another experiment was conducted using an abiotic MFC with its anode poised at a negative potential (ca. -200 mV Vs Ag/AgCl reference electrode) using a Potentiostat-Galvanostat (PG 581, Uniscan Instruments, Buxton, UK) under similar conditions as described above (i.e. same temperature, electrode specifications and contact time). After the contact period, samples were taken from the flasks and analysed for phenanthrene using HPLC (Section 2.7.1.1). The adsorption isotherm was modelled onto the Brunauer, Emmett and Teller (BET) model of multilayer adsorption (equation (13), as modified for solutes in solutions (Ebadi et al., 2009)) for both polarized and non-polarized electrode.

$$q_e = q_m \frac{K_S C_e}{(1-K_L C_e)(1-K_L C_e+K_S C_e)} \quad \text{.....(13)}$$

(q_e = amount of adsorbate adsorbed on the solid surface, mg/cm^2; q_m = amount of phenanthrene corresponding to complete monolayer adsorption, mg/cm^2; K_s = equilibrium constant of adsorption for first layer, L/mg; K_L = equilibrium constant of adsorption for upper layers in BET isotherm, L/mg and C_e = equilibrium concentration of adsorbate in the liquid phase, mg L^{-1}).

2.6.1.3 Benzene degradation in MFC using different bacterial strains.

The influence of inoculum type on MFC performance was investigated using *S. oneidensis* MR1 14063 (S.O), *P. aeruginosa* NCTC 10662 (P.A), a coculture of *S. oneidensis* MR1 14063 and *P. aeruginosa* NCTC 10662 (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the coculture (MCO), anaerobic digested sludge with *S. oneidensis* MR1 14063 (MCS), anaerobic digested sludge with *P. aeruginosa* NCTC 10662 (MCP) and lastly adapted anaerobic digested sludge, AMC (Table 2.4). Each inoculum was 10 % v/v of the working volume of the anode (200 mL) while the anodic chamber (of 250 mL total capacity) had a

working volume of 200 mL which contained the minimal medium (as described in section 2.2), 100 mg L^{-1} organic substrate (pyruvate or glucose), 200 mg L^{-1} benzene and the inoculum (20 mL). For inoculum containing the mixture of two different culture, they were grown in different growth media individually as described in section 2.2 and the mixture prepared at the ratio of 1:1. The measured optical density (OD) of each inoculum used in this study using different strains combinations was 0.6 at 600 nm by following a method described in section 2.7.2. Also, in each treatment, four controls were employed namely; an abiotic MFC, disconnected MFC, non-MFCs (anaerobic control) and MFC with no benzene present. Exactly 2ml of samples collected from the MFCs at every 12 h interval during MFC operations were analysed for benzene degradation efficiencies and COD removal efficiencies using methods described in sections 2.7.1 and 2.7.3. Power density measurement and polarisation readings were taken at peak voltage during MFC operation for all experiments (see section 2.7.4). Toxicity of samples (5 mL each) drawn from the MFCs at benzene concentration of 200 mg L^{-1}, before and at the end of MFC operations, were determined using microtox assay as described in section 2.7.6. MFC performances of all inocula were assessed based on their degradation performances and electrochemical performances. The system performance index, **K** was calculated as;

$$\mathbf{K} = xyz / 1000 \quad \text{(14)}$$

where x, y and z represents degradation rate (mg $L^{-1}h^{-1}$), maximum power density (mWm^{-2}) and COD removal (%) respectively. A high **K** value is an indication good system performance. All experiments were carried out in duplicate and the data

presented are means ± SD of duplicate tests**.** The experiment was carried out in dual chamber MFCs assembled and operated as described in Section 2.5.1.

Table 2.4: Summary of biocatalyst(s) used in each experiment for benzene studies

	Strain used			**Inoculum type**
Experimental run	*P.aeruginosa*	*S.oneidensis*	Undefined mixed culture	
1	-	+	-	S.O*
2	+	-	-	P.A
3	+	+	-	CO (P.A & S.O)
4	-	-	+	MC
5	+	+	+	MCO
6	-	+	+	MCS
7	+	-	+	MCP
8	-	-	+[a]	AMC
9	-	-	-	Abiotic control

*Glucose (100 mg L^{-1}) was used as organic substrate in all experiments except for *S.oneidensis* (S.0) where pyruvate (100 mg L^{-1}) was used in lieu of glucose. [a] Adapted mixed culture, + means present, - means absent

2.6.1.4 Effect of organic substrate concentration on MFC performance

The effect of organic substrate concentration on MFC performance was determined using petroleum hydrocarbon mixture (i.e. 30 mg L^{-1} phenanthrene and 200 mg L^{-1} benzene) supplemented with minimal medium (as described in section 2.2) in MFCs inoculated with *S. oneidensis* and MFC-adapted mixed culture. The MFC-adapted mixed bacterial culture was taken from an already operating fed-batch MFC, previously inoculated with a culture of anaerobic digested sludge treating petroleum 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 (200 mL). The concentration of methanol used in the preparation of dissolved phenanthrene was 430 mg L^{-1}. Sodium pyruvate (for *S. oneidensis*) and glucose (anaerobic digested sludge) were used as organic substrate with ranging concentrations of glucose (50 mg L^{-1} to 1000 mg L^{-1}) and sodium pyruvate (50 mg L^{-1} to 2200 mg L^{-1}) respectively. The initial

concentrations of petroleum hydrocarbon mixture were kept constant (at concentrations above) throughout the set of experiments. The abiotic control (no inoculum added) experiments used in this work were cell-free MFCs with abiotic condition maintained by the addition of 200 mg L^{-1} mercury (II) chloride in order to account for abiotic losses (Ma et al., 2011). The same medium was used as the other experiments and MFCs were operated under the same conditions as the tests. Reactor performance was monitored in terms of power outputs, petroleum hydrocarbon and COD removal efficiencies (sections 2.7.1.1 and 2.7.3).

2.6.2 Studies on the robustness of MFC systems: Effect of different operating conditions and the interactive effects among some selected parameters (Objectives 2 &3)

This section described studies presented in chapter 4; all experiments described under this section were carried out in duplicate, or otherwise stated and the data presented are means ± SD of duplicate tests. Secondly, for all experiments described under this section, exactly 5 mL of samples collected from the MFCs at every 24 h interval during MFC operations were analysed for petroleum hydrocarbons degradation efficiencies and COD removal efficiencies using methods described in sections 2.7.1 and 2.7.3. Power density measurement and polarisation readings were taken at peak voltage during MFC operation for all experiments (see section 2.7.4). Other electrochemical parameters that were also monitored during MFC operations include coulombic efficiency, current density, internal resistance following methods described in section 2.7.4. The inoculum source for dual chamber MFC system was the hydrocarbon-adapted mixed bacterial culture from an already operating fed-batch MFC system treating simulated petroleum hydrocarbon wastewater as described earlier. The biomass was centrifuged, washed twice with sterile phosphate buffer

before being introduced into the anode chamber at approximately OD_{600nm}= 0.6 per anode volume. The adapted mixed microbial culture was introduced into the MFC units at 10 % of the total reactor working volume. Dual chamber MFCs were assembled and operated as described earlier in section 2.5.1 for all experiments in this section. The work described in chapter 4 was conducted in several separate experiments as indicated below.

In one set of tests, **long-term fed-batch operation of the MFC system** containing hydrocarbon-contaminated wastewater was conducted at 30℃ for 60 days (i.e. over six cycles). The anolyte medium consisted of 30 mg L^{-1} phenanthrene, 200 mg L^{-1} benzene and minimal medium (as described in section 2.2). The concentration of methanol used in the preparation of dissolved phenanthrene was 430 mg L^{-1}. A cycle was deemed to have ended when MFC voltage fell below 5 mV and a new cycle was immediately initiated by removing 90 % of the anode content; subsequently, the anode chamber containing 10 % v/v of preceding cycle (as inoculum) was replenished with fresh anolyte medium. A similar operation was performed for a negative control (abiotic), one devoid of inoculum.

The **effect of operating temperature on MFC performance** was also evaluated by changing the temperature of the MFC system in the range of 20, 30 40 and 50℃. The **effect of salt concentration** on degradation efficiency of the hydrocarbons and power generation in the anode of the MFC system was investigated using anolyte feed solutions containing NaCl ranging from 0 ,0.5, 1.5, 2.0 to 2.5 % (w/v). For the salinity experiment, a temperature of 30℃ was maintained throughout at all salt concentrations tested. The abiotic controls used in all sets of experiments described above (i.e. the effects of operating temperature and salt concentration on MFC performance) contained the same anolyte medium as the tests but were not seeded

with the hydrocarbon-adapted microbial consortium. The abiotic controls were operated under the same conditions as the tests but without microbial inoculum added. The **effect of two redox mediators** namely; Riboflavin and Anthraquinone-2-Sulfonate (AQS) on MFC performance was also investigated. For this experiment, 30 µM redox mediator was added to the anolyte medium (as described above) from filter sterilised 10 mM synthetic redox mediator stock solutions. and MFCs were run in a batch mode at operating temperature (30℃). The control used was a MFC identical to the test but without any redox mediator present. In another study, the **effect of external resistance on MFC performance** was investigated. Separate experiments were carried out at different applied external resistances 100 Ω, 1000 Ω, 10 kΩ and 100 kΩ across external circuit of MFCs fed with petroleum hydrocarbons mixture. An open circuit control was ran in parallel with all experiments for comparison purposes. Two mL samples were taken from all the reactors at the end of MFC operation and biomass yields determined using optical density method as described in section 2.7.2.

The **effect of chemical catholytes** as a cheap alternative terminal electron acceptor on cathodic MFC performance was investigated. The anolyte medium composition used in this study was similar to those mentioned above (in section 2.6.2). Three chemical catholytes (which included potassium bromate, potassium persulfate and calcium hypochlorite) at the same concentration of 1000 mg L^{-1} (pH 2) were tested. Potassium ferricyanide and platinum coated cathode were used as baseline controls for performance-based comparison. A dual chamber MFCs was assembled and operated as described earlier in section 2.5.1.

The **influence of non-ionic surfactants (namely Tween 80 and Triton X100)** on phenanthrene degradation in MFCs inoculated with the adapted microbial

consortium (section 2.3) was investigated by adding various concentrations of surfactants, ranging from 20, 200 to 600 CMC (critical micelle concentration) to the anolyte medium. The CMC values at 30℃ for Tween 80 and Triton X100 are 11 mg L^{-1} and 138 mg L^{-1} respectively. The anolyte medium used in this study contained 30 mg L^{-1} phenanthrene and minimal medium as described above in other experiments. . The concentration of methanol used in the preparation of dissolved phenanthrene was 430 mg L^{-1}. Control experiments were performed under same conditions as the tests without surfactants to account for the effect of any other possible factors on bacterial growth. Samples (5 mL) were taken at the start and end of MFC batch operation and the phenanthrene concentrations were determined subsequently using HPLC as described in section 2.7.1.1. Toxicity of samples (5 mL each) drawn from the MFCs, at the start and end of MFC operations, were determined using microtox assay as described in section 2.7.6.

The **effect of substrate concentrations** on degradation performance and MFC performance was investigated. Benzene, phenanthrene and the mixture of both compounds were target substrates under investigation in this study. Benzene and phenanthrene concentrations used in this study were 200, 500, 1000, 1500 to 2000 mg L^{-1} and 10, 20, 30, 50 to 100 mg L^{-1} respectively. Adapted mixed culture collected from a running MFC was used as inoculum at 10 % v/v of the reactor's working volume (section 2.5.1). The concentration of methanol used in the preparation of dissolved phenanthrene was 350, 430, 790, 1350 mg L^{-1} for corresponding phenanthrene concentration in an increasing order. There were two controls employed namely; abiotic control and control without petroleum hydrocarbon. Mercury (II) chloride was added to the abiotic control to ensure that microbial activities were suppressed (Ma et al., 2011) .

The interaction of critical factors (such as redox mediator, salinity and external resistance) in aqueous MFCs was investigated using response surface methodology (RSM). RSM is a statistical method based on the multivariate non-linear model that is useful in studying interactions of various parameters affecting the process (Mundra et al., 2007; Chou et al., 2010). For the work described in chapter 4 (section 4.2.10), a RSM design, central composite face centered design (CCFD), employed had three independent variables viz., salinity (A), external resistance (B), redox mediator (riboflavin) (C). Selection of these three independent variables was based on good performance results obtained from previous studies on the effect of individual operating factors on MFC performance (chapter 4). Selection of three levels of each independent variable (salinity: 0.5, 1.0, 1.5 % w/v NaCl; external resistance: 0.1, 1, 100 kΩ; redox mediator: 10, 30, 50 µM) yielded a total of 20 different experimental runs in order to explore the effect of independent variables on the reactor's response within the region of investigation. The experimental matrix for central composite face design for general optimization is presented in Table 2.5. Three response factors measured were COD % efficiency, power density and total petroleum hydrocarbon, (TPH) % removal. The statistical software Design Expert 9.0.7 (Stat-Ease Inc., Minneapolis, USA) was used to evaluate the analysis of variance ($P < 0.05$) to determine the significance of each term in the fitted equations and to estimate the goodness of fit in each case. The levels and the independent variables were selected based on preliminary study results and literature. The system was operated in a batch mode for at least 8 days under each condition at a fixed load (1 kΩ, unless stated otherwise). Dual chamber MFCs were assembled and operated as described earlier in section 2.4.1. About 5ml of samples collected from the MFCs at the end of MFC operations were analysed for

petroleum hydrocarbon and COD removal efficiencies using methods described in sections 2.7.1 and 2.7.3. Power density measurement were taken at peak voltage during MFC operation for all experiments (see section 2.7.4). MFC reactors were maintained at 30 ± 3℃ by means of a Stuart 160D incubator (Fisher Scientific, UK) and all the experiments were carried out in duplicates.

Table 2.5: Experimental matrix for central composite design (CCD) for all experiments.

Run	Point type	Factors		
		A – Salinity (%)	**B – External resistance (Ω)**	**C– Redox Mediator, Riboflavin (µM)**
1	Factorial	1.50	100000	10
2	Factorial	1.50	100	10
3	centre	1.00	1000	30
4	Factorial	0.50	100	50
5	Axial	1.00	1000	50
6	Axial	1.00	100	30
7	centre	1.00	1000	30
8	Factorial	0.50	100	10
9	centre	1.00	1000	30
10	centre	1.00	1000	30
11	Factorial	0.50	100000	50
12	Axial	1.00	1000	10
13	Factorial	1.50	100000	50
14	Factorial	0.50	100000	10
15	centre	1.00	1000	30
16	centre	1.00	1000	30
17	Axial	1.50	1000	30
18	Axial	1.00	100000	30
19	Axial	0.50	1000	30
20	Factorial	1.50	100	50

2.6.3 Studies on MFC applications to treatment of petroleum contaminated groundwater system and refinery wastewater using two different tubular MFC designs (Objective 4)

The treatment of petroleum hydrocarbons using two chamber tubular MFCs was investigated using hydrocarbon adapted microbial mixed culture (section 2.3) as inoculum for the work presented in chapter 5. The experimental set-up consisted of four identical tubular MFC units operating in continuous flow mode, two of which were duplicate tests while the other two were an open-circuit control and a non-MFC (or anaerobic) control. These MFC units were operated as previously described in section 2.5.2. The tubular MFCs were operated in a continuous mode at different HRTs under ambient temperatures (14 - 23℃) by feeding both chambers at the same flow rates (as dictated by different HRT regimes) from two different feeding tanks containing petroleum hydrocarbon contaminated wastewater and bromate solution each simultaneously. The petroleum hydrocarbon contaminated wastewater was composed of minimal medium supplemented with 30 mg L^{-1} phenanthrene and 200 mg L^{-1} benzene. The concentration of methanol used in the preparation of dissolved phenanthrene was 430 mg L^{-1}. HRTs were decreased stepwise from 10, 5, 2.5 days to 2 days only after at least a throughput of three reactor volumes has been achieved. The stability and subsequent recovery of MFC systems during extreme nutrient conditions were also investigated. Two nutrients conditions considered were low substrate (50 µg L^{-1} each for phenanthrene and benzene) conditions and high substrate (1500 mg L^{-1} benzene and 100 mg L^{-1} phenanthrene) conditions. The concentration of methanol used in the preparation of dissolved phenanthrene for low and high substrate conditions was 0.95 mg L^{-1} and 1500 mg L^{-1} respectively. MFCs were operated continuously under those extreme substrate level conditions ,at a

constant HRT of 10 days. Ten (10) mL of th samples were drawn from all reactors and feeding tanks at 12 hrs intervals throughout about 160 days of MFC operation and reactor performance was monitored in terms of petroleum hydrocarbon and COD removal efficiencies (sections 2.7.1.1 and 2.7.3).

In another study, a different MFC design, the tubular single chamber MFC was employed in the treatment of petroleum hydrocarbon-containing wastewater at different HRTs in a continuous mode operation (section 5.2.2). The design and operation of this single chamber MFC reactor have been described in section 2.5.2 while for this study, three MFC units of same design 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, 20 to 30 h at ambient temperatures ranging between 15℃ and 25℃ during MFC operations. HRT was changed every three weeks of continuous operation (having achieved at least a throughput of three reactor volumes). The influent in the feeding tank was made of minimal medium (section 2.2) supplemented with 30 mg L^{-1} phenanthrene and 200 mg L^{-1} benzene. The concentration of methanol used in the preparation of dissolved phenanthrene was 430 mg L^{-1}. The potential for MFC recovery from shock-load effect (in terms of reactor performance) and ability to withstand a sudden shock-load of petroleum hydrocarbon mixture (100 mg L^{-1} phenanthrene and 1500 mg L^{-1} benzene) in were also 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 th 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 (sections 2.7.1.1 and 2.7.3).

2.6.4 Studies on MFC applications to bioremediation of phenanthrene-contaminated soil using a lab-scale tubular MFC (Objective 5)

Experiments were designed to investigate the bioelectrochemical treatment of phenanthrene-contaminated soil using a soil MFCs described in section 2.5.3. For the study presented in chapter 6, there were two rectangular PVC storage containers as shown in Figure 2.4A with each container having two MFC units installed. The original soil (previously described in section 2.4) 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 (at room temperature) for partial aging and enrichment of the indigenous microbial population. In one of the storage PVC container, a non-ionic surfactant Tween 80 (500 mg L^{-1}) was mixed with soil to examine if the addition of surfactant would improve desorption and transfer of phenanthrene from soil toward the anode area of influence. Waterlogged soils under open-circuit and non-MFC conditions were used as the control and the blank (which reflected the baseline natural attenuation), respectively. The catholyte (1000 ppm bromate solution at pH 5) was replaced three times a month during the operational period. Soil samples (about 10 g wet soil wt) were routinely extracted from the top, middle, and bottom of the soil layer at varying distances from the anode (i.e. 2 cm, 4 cm and 8 cm). The composite soil samples were analysed for petroleum hydrocarbon degradation efficiencies, bromate removal efficiencies and TCOD removal (sections 2.7.1 and 2.7.3). Physicochemical of soil samples such as pH, total dissolved solid

(TDS) and electrical conductivity (EC) (section 2.7.2). Power density measurements were taken twice weekly throughout the 60 days period of MFC operation (section 2.7.4). Toxicity of soil samples were determined using microtox assay as described in section 2.7.6. Cyclic voltammetry analysis was done on day 10 and 30 for all MFC reactors as described in section 2.7.5.

The distance from the anode's outer surface to the location where phenanthrene concentrations are lower than that in the baseline control is known as radius of influence, ROI. Indigenous microbes remained in all systems without any external inoculation. All reactors were operated for 60 days at room temperature (23 ± 5℃) protected from sunlight or light irradiation.

2.7 Analytical methods

2.7.1 Chemical analysis

2.7.1.1 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^{TM} 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 (at the end of each cycle) 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 using 25 mL sterile syringe) 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 amount of petroleum hydrocarbon present on the electrode was determined by soaking the anode electrodes in 10 mL methanol, at the end of each experiment, on a shaker for 1 h at 200 rpm. Aliquots were transferred into 2 mL eppendorf, immediately followed by centrifugation at 13.2 x g for 10 mins. 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., 2010a). Degradation efficiencies and rates were determined based on the remaining petroleum hydrocarbons in solution and those adsorbed on the electrode at the end of MFC operation. The degradation performance can be expressed as degradation efficiency and degradation rate. The degradation efficiency was calculated as follows:

$$\textbf{Degradation efficiency}\ (\%) = \frac{D_i - D_f}{D_i} \times 100 \quad (15)$$

where D_i and D_f are initial and final hydrocarbon concentrations respectively.

$$\textbf{Degradation rate}\ (\text{mg L}^{-1}\ \text{h}^{-1}) = \frac{D_i - D_f}{D_i t} \times 100 \quad (16)$$

where D_i and D_f are initial and final hydrocarbon concentrations respectively; t is time taken for each experiment when the voltage had dropped below 5 mV.

In order to determine the amount of phenanthrene present in the solid phase of the soil MFCs (for the work reported in chapter 6), 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 (1997) 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.

2.7.1.2 Determination of bromate removal in MFCs

In the work described in chapters 4, 5 and 6, the amount of bromate removal was determined quantitatively by using spectrophotometric method as described by Emeje et al (2010). One mL of freshly prepared 0.5 % w/v potassium iodide (KI) solution in 0.1 M hydrochloric acid (HCl) was added to 1 mL of the sample; the mixture was then vortex 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 (ranging from 0 to 1000 mg L^{-1}) were prepared by the dilution of potassium bromate ($KBrO_3$) solution (Sigma Aldrich, UK) with deionised water for calibration curve generation (see Appendix 2) used to quantify the concentration of bromate in the samples from the absorbance readings. The concentration of bromate consumed was expressed as percentage bromate removal.

The percentage bromate removal was calculated as follows;

$$\textbf{Percentage bromate removal}\ (\%) = \frac{Br_i - Br_f}{Br_i} \times 100 \quad \text{..... (17)}$$

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

2.7.2 pH, conductivity, total dissolved solid (TDS) and bacterial optical density (OD) measurements

The pH of the anodic medium during MFC operations and at the end of each batch-cycle was measured using a Mettler Toledo MP220 pH meter (UK). pH changes in the cathode chamber of MFCs containing bromate as catholyte was also monitored. For the work described in chapter 6, conductivity and TDS measurements were taken using an Oakton PC-700 (Oakton Instruments, UK) conductivity probe. The TDS and conductivity of soil were measured in a 1:5 (w/v) soil: deionized water mixture. For the work presented in chapters 3 and 4, biomass yield was expressed as bacterial OD. One ml samples were taken from MFCs and broth cultures and their bacterial OD was measured by a UV-Vis spectrophotometer M 6300 model (Jenway Staffordshire, UK) at 600 nm.

2.7.3 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.

For soil samples (in the work reported in chapter 6), 2 g of soil sample was diluted with 10 mL deionized water to measure total COD (TCOD). 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;

$$\textbf{COD}\ (\text{mg L}^{-1}) = (K_b - K_s) \times DF \times M \times 8000 \quad \text{.....(18)}$$

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:

$$\textbf{Percentage COD removal}\ (\%) = \frac{COD_i - COD_f}{COD_i} \times 100 \quad \text{.....(19)}$$

where COD_i and COD_f are initial COD and final COD values respectively for each experiment when the voltage had dropped below 5 mV.

$$\textbf{COD removal rate}\ (\text{mg L}^{-1}\ \text{h}^{-1}) = \frac{COD_i - COD_f}{COD_i t} \times 100 \quad \text{.....(20)}$$

where COD_i and COD_f are initial COD and final COD values respectively and t is time taken for each experiment when the voltage had dropped below 5 mV.

2.7.4 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 intervals 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;

$$P = \frac{I \times E}{A} \quad \text{.....(21)}$$

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

$$\mathbf{CE}\ (\%) = \frac{\int I\, dt}{C_t} \times 100 \quad \text{..... (22)}$$

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).

2.7.5 Cyclic Voltammetry analysis

In the work described in chapter 6, 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 anode served as the working electrode, cathode as the counter electrode and a Ag/AgCl electrode (BASi, Germany, 4M KCI, +196 mV versus standard hydrogen electrode (SHE) at 25℃) in a sealed chamber was used as a reference electrode. The bioelectrochemical cell was kept at 30℃ unless otherwise stated. The device was operated remotely through a personal computer (PC) using UIE Chem v3.54 software.

2.7.6 Bioluminescence toxicity assays

In the work described in chapter 3, 5 and 6, 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 (see Table 2.2) 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 % w/v NaCl solution before use in the toxicity assay. Toxicity assessments were conducted for samples drawn from all reactors before and at the end of MFC operations. All samples analysed were centrifuged at 13.2 x *g*, filtered through 0.22 µm PTFE filters to remove suspended biomass. Exactly 2 % w/v 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 (section 2.7.3).

In another study (described in chapter 4), the half-maximal effective concentration (EC_{50}) was expressed as a percentage of the initial concentrations of test samples (since the toxic components were unidentifiable). In order to facilitate data interpretation, toxicity results were converted to toxic units (TU) using the following equation:

$TU = 100 / EC_{50}$ .....(23)

2.7.7 Microbial community analysis

Microbial community analysis was carried out by amplification and analysis of the 16s ribosomal DNA (rDNA) of microbial population in the samples using PCR-DGGE.

2.7.7.1 Sample preparation, DNA extraction and PCR amplification

Two samples (5 mL each) collected from an MFC fed with simulated wastewater containing petroleum hydrocarbon mixture (operated in fed-batch mode) at the end of 60 days of MFC operation and were centrifuged at 6500 g for 10 mins. The total bacterial genomic DNA was extracted from the pellet using a Sigma Aldrich Gen-Elute bacterial DNA kit (based on manufacturer's instructions). The whole genomic

DNA isolates were verified using gel electrophoresis in 1 % (w/v) agarose gels stained with SYBR® Green I (Sigma Aldrich, UK) by incorporating 5 µL of 10 000X water-based stock reagent to a 50 mL agarose gel prior to PCR amplification. Electrophoresis was conducted in 1X Tris-Acetate EDTA (TAE) buffer at 200 V for 5 h at 60℃. The DNA isolates was amplified in an Eppendorf® Mastercycler PCR (UK) using the genomic DNA isolates as template and the following set of primers; F357- GC (5'- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG - 3') and R518 (5'- ATT ACC GCG GCT GCT GG - 3') primers (Sun et al., 2012).

The PCR reaction mixture (50 µL) contained the components given in Table 2.6. The amplified PCR products were verified using gel electrophoresis through 1.5 % (w/v) agarose gels for correct product size confirmation prior to DGGE microbial community profiling.

Table 2.6: Composition of the PCR reaction mixture used in this study

Reagents	**Quantity used (µL)**
2X PCR master mix (New England Biolabs, UK)	25
Nuclease free water (Promega, UK)	22
Forward primer (F357- GC)	1
Reverse primer (R518)	1
Whole genomic DNA template	1

The PCR was performed under conditions summarised in Table 2.7.

Table 2.7: PCR program used for the amplification of the total genomic DNA isolates

Component	Temperature (℃)	Time (min)	
Initial denaturation	95	4	
Denaturation	95	0.5	30 cycles
Annealing	58	1	
Extension	72	0.5	
Final extension	72	7	

2.7.7.2 DGGE profiling, DNA sequencing and phylogenetic analysis

Microbial community (DGGE) profiling was carried out using Bio-Rad D-Code universal mutation detection system (Bio-Rad Inc, USA) using 8 % (w/v) polyacrylamide gels (37.5:1 acrylamide:bis-acrylamide, gel stock solution; Sigma Aldrich, UK) with a denaturant gradient ranging from 30 % - 60 % across the gel (100 % corresponding to 7 M urea and 40 % deionised formamide), 1 x TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.3), containing 0.04 % (w/v) APS (ammonium persulfate; Sigma Aldrich, UK) and 0.02 % (v/v) TEMED (N,N,N',N'-tetramethylethylenediamine; Sigma Aldrich, UK). The solutions were cast into the glass plates using the Bio-Rad model-475 gradient delivery system. The components of the denaturing gel are listed in Table 2.8.

Table 2.8: Composition of the denaturing gels used in microbial community analysis

Component	**Quantity**		
	0%	30%	60%
Tris- acetate EDTA (TAE) buffer 50X stock	0.5 mL	0.5 mL	0.5 mL
Acrylamide (40 % 37.5:1 stock)	5 mL	5 mL	5 mL
Deionized Formamide	-	3 mL	6 mL
Urea	-	3.15 g	6.3 g
Glycerol	0.5 mL	0.5 mL	0.5 mL
Nuclease free water	To 25 mL	To 25 mL	To 25 mL

The PCR products were loaded into the wells with a tracker dye (6X concentrated, Promega, USA) and run in 1X TAE buffer for 16 h at 70 V and a constant temperature of 60℃. After the DGGE run was completed, the gel was stained with SYBR® Green I in 1X TAE for 10 mins and was visualised under UV- polarised light using a transilluminator table. Bands were excised from the gel using sterile scalpels and the cut-out gel blocks were incubated at 4℃ for 24 h in Eppendorf tubes containing 100 µL of nuclease free water.

The dissolved DGGE gel solution was used as the template for second round of PCR amplification using the bacterial primer set F-338 (5'- ACT CCT ACG GGA GGC AGC AG - 3') and R518 (Sun et al., 2012) in a Eppendorf® Mastercycler PCR following the PCR conditions shown in Table 2.7. The size of the PCR products were verified on 1.5 % (w/v) agarose gels in gel electrophoresis ran at the same conditions described earlier in section 2.7.7.1. The bands were excised from agarose gels and PCR products in the gels were later purified using a QiaQuick gel extraction kit (QiaGen, UK) following the product manual's extraction procedures. The

concentration and purity of the PCR products were also verified using a Nano-Drop spectrophotometer (ND 1000, Thermo Scientific, USA). The purified DNA was stored at -20℃. The DNA samples were sequenced at GATC Biotech, Germany.

The sequences obtained were analysed using the NCBI GenBank 16s rDNA gene fingerprint repository (http://blast.ncbi.nlm.nih.gov/Blast) using the basic local alignment search tool (BLAST). Phylogenetic analysis of the sequences was conducted using MEGA 6.06, molecular evolutionary genetics analysis tool with neighbour joining method (using 1000 bootstrap replicates) and distance matrix.

2.8 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). One - way analysis of variance (ANOVA) was used to compare MFC performances and degradation rates among all treatments. All ANOVAs showing significant differences were followed by post-hoc Tukey's comparison tests. Correlation analyses were also conducted (using Prism Graph Pad 5.0) to measure the degree of association between data collected. RSM, a statistical design experimental tool, was employed for the work described in section 2.6.2 and data processed using Design-Expert® Software Version 9.0 (Stat- Ease Inc., USA). The modeling of adsorption data was done in Microsoft Excel using the solver add-in.

CHAPTER 3

The degradation of substrates in the anode of dual-chamber MFCs.

3.1 Chapter overview

Microbial fuel cells (MFCs) have been proposed as having the potential for bioremediation of various contaminants (Aulenta and Majone, 2010; Mu et al., 2009; Mu et al., 2011; Bin et al., 2013). MFCs are unique in the sense that the microorganisms are able to transfer (or receive) electrons extracellularly to a solid material like an anode electrode. The use of oxygen as an indirect TEA would be expected to enhance hydrocarbon degradation compared to degradation via anaerobic respiration.

MFCs have been investigated in the bioremediation of hydrocarbons in model contaminated soil and groundwater systems but only a few studies have been undertaken (Mu et al., 2009; Luo et al., 2009; Morris et al., 2009; Wang et al., 2012b; Yan et al., 2012). Morris et al (2009) achieved 82 % diesel range organics (DRO) removal with an MFC over 21 days compared to an anaerobic incubated control, which achieved 31% removal. Rakoczy et al (2013) recently demonstrated benzene and sulphide removal (with removal efficiencies of 80 % and 90 % respectively) from contaminated groundwater in a dual-chambered MFC over a period of 770 days. Most studies investigating the feasibility of treating petroleum hydrocarbons in wastewater, sediments and groundwater systems used undefined mixed cultures or indigenous microbes as inocula (Chandrasekhar and Venkata Mohan, 2012; Mu et al., 2009; Li et al., 2012; Dou et al., 2008). Most of the researchers recorded prolonged experimental durations ranging from 20 - 240 days. Unfortunately, this could limit the potential application of this technology in real scenarios.

The use of pure or defined co-cultures in the presence of organic substrates could reduce the period required to degrade hydrocarbons. In the case of co-cultures, there could be potential for synergistic utilisation of the metabolic pathways from the microorganisms involved (Bader et al., 2010). Such synergy may involve one organism reducing available oxygen in the anode thus enhancing growth of another microaerophillic microorganism or strict anaerobe. Alternatively, by-products of one microorganism may be used by another microorganism as substrate, redox mediator, surfactant etc. The aforementioned two examples may be representative of the co-culture of *Pseudomonas aeruginosa* and *Shewanella oneidensis.* Mixed cultures were reported to show good process stability in MFCs (Pham et al., 2006); the two strains could have potential for bioaugmentation of mixed cultures used in MFCs.

Carbon materials used in MFCs as anodes can adsorb petroleum hydrocarbons in aqueous or particulate media (Yuan et al., 2010). Therefore, they could play an essential role in initial removal of hydrocarbons from any medium during MFC operation. However, this adsorption effect has rarely been quantified in the literature.

This chapter reports on the biodegradation of phenanthrene and benzene in a microbial fuel cell using a range of inocula (*Shewanella oneidensis, Pseudomonas aeruginosa*, mixed cultures and combinations thereof). The effect of organic substrate concentration on MFC performance was also investigated. The degradation rates, degradation efficiency, power production and the assessment of phenanthrene's adsorption on a carbon electrode were determined.

3.2 Results and Discussion

3.2.1. Adsorption of phenanthrene onto the carbon anode

Adsorption studies revealed that the anode (carbon) electrode had a high affinity for phenanthrene with a maximum adsorption capacity of 0.45 $mgcm^{-2}$ (within the range of concentrations tested). The adsorption isotherm (Figure 3.1) was of Type II, which is a typical of non-porous solids or solids with large pores (macropores of size >50 nm). Adsorption of phenanthrene appears to proceed layer by layer with chemisorption (which follows the Langmuir isotherm) taking place in the first instance (up to the inflection point A) and physiosorption (to form multi-layers) taking place later-on (point B) (Carmody et al., 2007). The adsorption isotherm has been modelled onto the Brunauer, Emmett and Teller (BET) model of multilayer adsorption as described in section 2.6.1.2 and it was found that q_m = 0.088$mgcm^{-2}$, K_s = 1.38 L/mg, K_L = 0.018 L/mg for the non-polarized electrode.

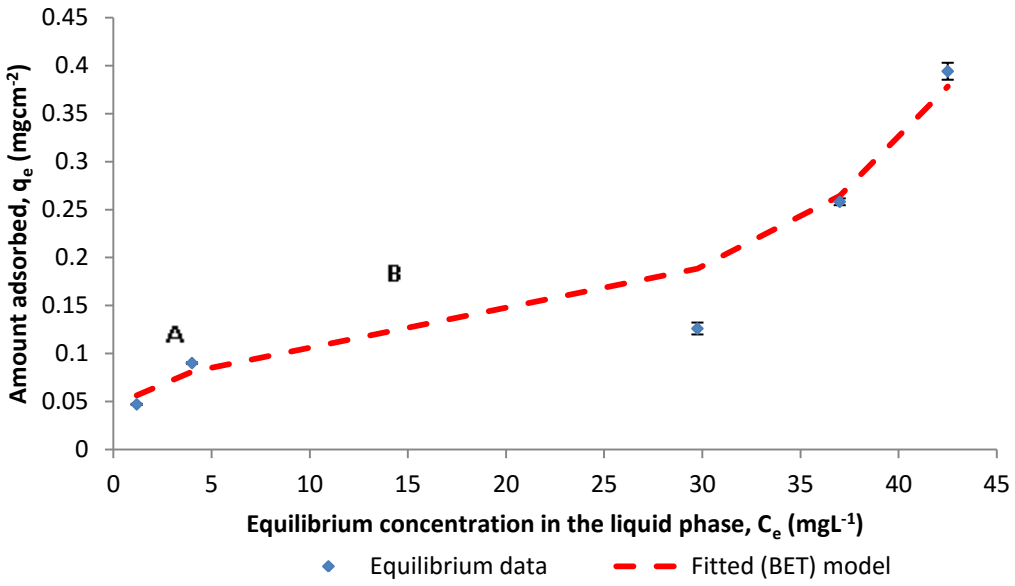


Figure 3.1: Adsorption isotherm of phenanthrene onto carbon felt [pH = 7; contact time = 180 mins; agitation speed: 0 rpm; adsorbent's surface area = 40 cm^2, T = 30℃]. The error bars represent the SD of the mean.

For the polarized electrode, the corresponding values were q_m = 0.079 mgcm^{-2}, K_s = 0.34 L/mg and K_L = 0.011 L/mg. Notably, the adsorption data for the polarized electrode were lower than the non-polarised electrode as observed in this study. A possible explanation for this could be the negative anode potential leading to increased repulsive forces between the electrode's surface and the partially negatively charged phenanthrene molecules thereby limiting affinity of the phenanthrene molecules for the electrode (Pepprah, 2007).

The shape of the adsorption isotherm is related to the texture of the solid (pore shape and size, percent porosity, specific surface area etc.). The isotherm observed for phenanthrene on carbon felt indicates that the electrode is porous with adsorption taking place in two stages: a fast stage followed by a slow one (Yuan et al., 2010). Various mechanisms may contribute to adsorption of phenanthrene onto the carbon electrode e.g. weak intermolecular forces such as π-π interactions, H-bonding and electron donor- acceptor interactions (Yuan et al., 2010).

Walters and Luthy (1984) demonstrated that PAHs adsorbed strongly onto porous carbon materials in preference to soils, sediments and other suspended organic matters, suggesting the suitability of carbon materials for co-localisation or temporary removal of PAHs (and BTEX) compounds from aquatic environments (Zhang et al., 2010a; Yuan et al., 2010). Adsorption of petroleum hydrocarbons by the anode in MFCs was not considered in the work of Morris et al (2009) and Wang et al (2012b). The fact that the carbon-felt electrode is a good adsorbent for phenanthrene may suggest its use in treatment of petroleum hydrocarbons in both liquid and particulate systems. It is also suggested that kinetic studies of hydrocarbon degradation in MFC studies ought to include amounts adsorbed on the electrode

instead of relying only on what is in solution. The question of whether adsorption of hydrocarbons on anodes interferes with electron transfer is discussed in section 3.2.3.

3.2.2 Phenanthrene biodegradation in MFCs

3.2.2.1 Influence of inoculum type on degradation of phenanthrene

The degradation of phenanthrene for different inocula is shown in Figure 3.2. The degradation rates for phenanthrene using different inocula were determined based on their total concentrations (phenanthrene remaining in the aqueous solution and that adsorbed onto carbon electrode after the experiment) in comparison to the starting concentration. All the seven inocula (in connected MFCs) showed very high potential for phenanthrene degradation with minimum degradation efficiency of 97 % compared to the non-MFC (anaerobic) controls (Figure 3.3B). Abiotic loss in abiotic control might be due to loss resulting from adsorption of phenanthrene on glassware used for the experiments (Huang et al., 2011). The marked differences in degradation efficiency between the connected MFCs and the disconnected MFCs or non-MFCs (in all inoculum types) indicated an enhancement of phenanthrene degradation via electron transfer through a temporary electron acceptor (i.e. the anode) to the cathode where oxygen is used as terminal electron acceptor (Figure 3.3B). Notably, phenanthrene removal in disconnected MFCs was much higher than anaerobic controls (non-MFCs); this could be attributed to oxygen intrusion from the cathode (leading to aerobic degradation) and/or sorption of phenanthrene on the electrode possibly improving microbe-substrate interaction. Similar observations were made by Huang et al (2011) in a soil MFC. They suggested that the electrons produced by the oxidation of organic carbon in the open-circuit MFC might be transferred from the anode to the cathode via an internal soil matrix, such as bacterial

nanowires and conductive minerals, resulting in higher COD and contaminant (phenol) removal in the open-circuit MFC compared with those in non-MFC.

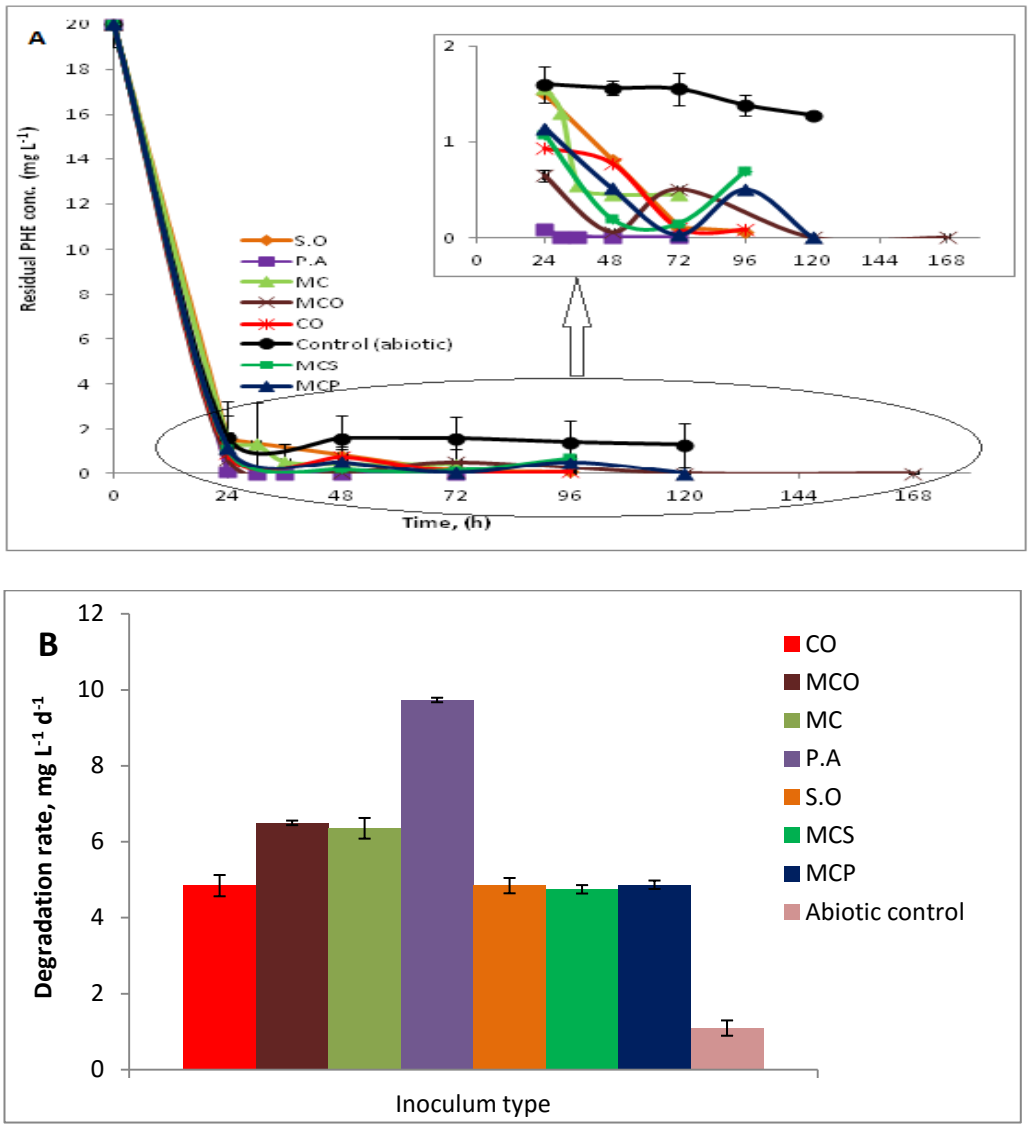


Figure 3.2: (A) Phenanthrene (PHE) concentration in the bulk anode solution as a function of time and inset: A clearer picture of the plot of residual phenanthrene concentration against incubation time between 24 and 170 h (B) phenanthrene (average) degradation rates by different inocula used. The error bars represent the SD of the mean

A marked variation in the phenanthrene biodegradation rate was observed as a function of inoculum type during the MFC operation. *P.aeruginosa* (P.A) gave the highest degradation rate of 9.73 mg L^{-1} d^{-1} followed by MCO and MC while the anaerobic sludge with *S.oneidensis* (MCS) gave the lowest degradation rate of 4.84 mg L^{-1} d^{-1}. Notably, there was no statistically significant difference ($p<0.05$) among

CO, SO, MCS and MCS in terms of their degradation performances. The two strains, *P.aeruginosa* and *S.oneidensis MR1* may possess PAH degrading enzymes that enabled them to biodegrade phenanthrene. *Pseudomonas* species have been reported by several authors to have a potential to degrade PAHs compounds (Ma et al., 2011; Nasseri et al., 2010). Ma et al (2011) successively isolated *P.aeruginosa strain* PAH-1 which had the ability to anaerobically degrade phenanthrene with anthraquinone-2, 6-disulfonate (AQDS) as the sole electron acceptor. The authors reported 56.7 % phenanthrene removal in the presence of a co-substrate, fructose. *Pseudomonas* species have also been found in MFC anodes and can be classified as electrochemically-active bacteria (Logan, 2008). They respire anaerobically via the production of phenazines and pyocyanin, electron shuttling compounds, which aid transfer of electrons to the anode (Logan, 2008). These redox shuttling compounds aid in facilitating enhanced microbial oxidation of organic compounds like phenanthrene via electron transfer to the anode. Since these redox electrons shuttling compounds enhance electron transfer, high degradation rates and power densities would be expected. High degradation rates were observed as presented in this study. However, due to large internal resistances, the power density appears to be very small. *P. aeruginosa* also secretes biosurfactants that may be expected to solubilize both suspended phenanthrene in anolyte and the phenanthrene adsorbed on the anode. Higher degradation rates observed with *P. aeruginosa* (relative to other inocula tested) might be due to the synergistic effect of its PAH degrading enzymes, biosurfactant self-production and the involvement of soluble shuttlers for the redox powers.

Anaerobic biodegradation of phenanthrene has previously been reported to occur via carboxylation followed by cleavage of the aromatic ring putatively at the K- region of the phenanthrene ring (Meckenstock et al., 2004). In this study, a decrease in pH (from 7 to 5.8) was observed at the end of MFC operation, indicating that some acidic intermediates were probably produced during the phenanthrene degradation for all inocula tested. However, due to the range of inocula tested and the presence of other organic substrate, identification of these acidic metabolites becomes complex and time consuming.

Degradation efficiencies recorded in this study are higher than those reported in the literature (as shown in Table 3.1) where other alternative electron acceptors (e.g. nitrate and sulphate) were used to enhance anaerobic degradation of certain PAH compounds. This is the very first report that demonstrated that pure strains such as *P.aeruginosa* and *S.oneidensis* can oxidise phenanthrene in a microbial fuel cell. Morris et al (2009) investigated enhanced anaerobic biodegradation of diesel using MFCs and subsequently conducted microbial community analysis in order to identify the microorganisms that catalysed the anodic reactions. *S.oneidensis* was found among the electrochemically active bacteria found in the anode of the MFCs.

The degradation rate obtained for co-culture (CO) (Table 3.1) was significantly lower than that for *P. aeruginosa* ($p<0.05$). This implies that the degradation performance of *P.aeruginosa* in the co-culture (CO) was inhibited, suggesting possible negative microbial interactions between the two microorganisms under tested conditions. The reason for this is not fully understood and requires further investigation. One possible explanation for this could be due to differences in the

redox conditions at which both strains thrive. *S.oneidensis* has been reported to thrive under anaerobic conditions more than *P.aeruginosa*; *S.oneidensis,* a dissimilatory metal-reducing bacterium, can couple metal reduction with their metabolism whereas *P.aeruginosa* cannot (Logan, 2008; Lovely, 1993). As a result, there could be a possible microbial population shift occurring within the anode chamber, especially on the anode biofilm when both strains are used as co-culture (CO i.e. PA & SO). Biofilms are formed on the anode as a result of the complex interactions among microbial population especially anode loving electrogenic bacterial species.

Table 3.1: Comparison of phenanthrene degradation rates for different inocula using different electron acceptors.

Electron acceptor	**Inoculum**	**Degradation rate (mg L^{-1} d^{-1})**	**Reference**
Nitrate	Marine sediment	0.59	Rockne and Strand, 1998
Sulfate	Marine sediment	0.02	Coates et al., 1996
Sulfate	Marine sediment	0.03	Coates et al., 1997
Carbon-felt	MC	6.35	
electrode[a]	P.A	9.73	
	S.O	4.84	This study
	CO	4.89	
	MCO	6.50	
	MCP	4.93	
	MCS	4.88	

[a] Carbon-felt electrode used in an MFC as anode.

In this study, we have demonstrated that *P.aeruginosa* gave a high degradation rate and biodegradation efficiency of 9.73 mg L^{-1} d^{-1} and 98 % respectively in a MFC reactor. It could potentially be used for bioaugmentation purposes. Bioaugmentation

involves the introduction of foreign strains with the metabolic capability to degrade the hydrocarbons of interest in an environment where the indigenous microbes are incapable and is one of the bioremediation techniques that have been used in field applications (Thompson et al., 2005). Nasseri et al (2010) reported the influence of bioaugmentation on biodegradation of phenanthrene-contaminated soil in a bio-slurry reactor. They demonstrated that the bioaugmented bioreactor (using *P.aeruginosa*) showed higher degradation efficiency of over 85 % compared to 17 % observed in a non-augmented reactor (indigenous microorganisms). Others authors such as Hamdi et al (2007) and Gao et al (2006), all reported enhanced PAHs biodegradation with the aid of bioaugmentation. Our results suggest *P.aeruginosa* as a possible choice for enhanced bioremediation of PAHs in contaminated environments.

3.2.2.2 Role of adsorption in biodegradation of phenanthrene during MFC operation

Phenanthrene concentrations in the bulk solution for all seven inocula decreased rapidly by about 90 % within 24 h followed by a gradual decrease afterwards (Figure 3.2A). The sharp decrease in all cases observed was attributed to phenanthrene adsorption by the carbon felt electrode. The disappearance of phenanthrene from solution appears to occur via two stages: the first stage (fast) is possibly controlled by adsorption while the second stage (slow) depends mainly on microbial action. Zhang et al (2010a) and Xia et al (2010) reported that microorganisms could degrade aromatic hydrocarbons on an electrode and in solution but initially the hydrocarbons were adsorbed. Adsorption of hydrocarbons on carbon electrodes appears to be a good thing in this case as it negates mass transfer resistances of substrate/redox mediator from the bulk solution to the microorganisms present in anode biofilm in

the MFC reactors. The mechanism of microbial degradation of adsorbed phenanthrene on the electrode is not fully understood.

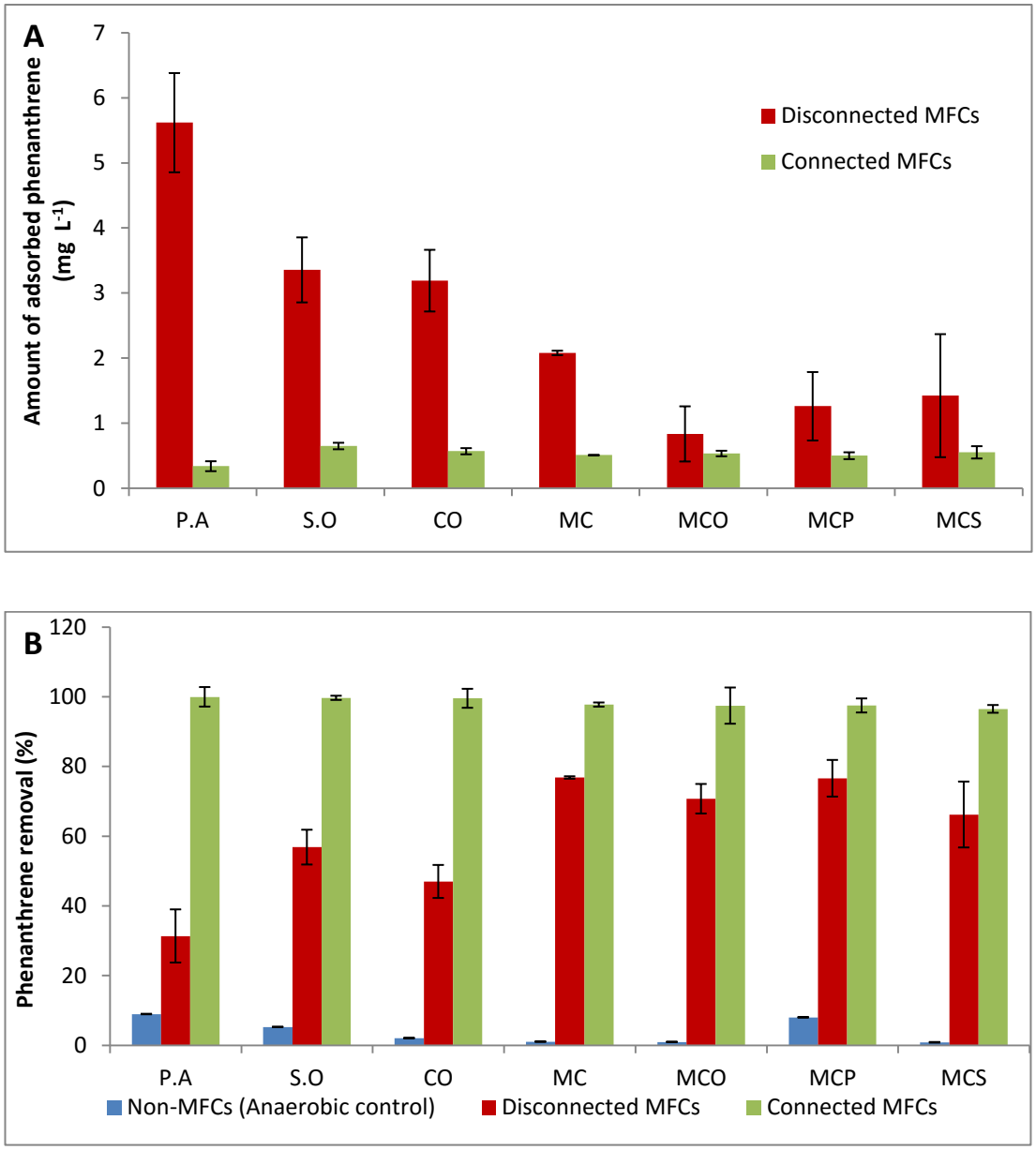


Figure 3.3: (A) Amount of phenanthrene adsorbed on the electrode after treatment and (B) phenanthrene removal by different inoculum types. Values are means of duplicate measurements ± SD. *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS) and lastly anaerobic digested sludge with *P. aeruginosa*, MCP.

Phenanthrene may desorb from the electrode followed by biodegradation or the biodegradation process may occur directly on the electrode (Velvizhi and Venkata Mohan, 2011). Considering the fact that the microbes used are electrode respiring bacteria, electrons produced as a result of substrate utilisation could be transferred either directly, or through any mediated process, that facilitates electron transfer (Chandrasekhar and Venkata Mohan, 2012). Figure.3.3A indicates that phenanthrene adsorbed on the electrode was probably utilised by anode-respiring bacteria as the amount adsorbed in connected MFCs was significantly different ($p<0.05$) from that adsorbed on electrodes in disconnected MFCs. This suggests that MFC technology could enhance phenanthrene degradation in contaminated anaerobic environments.

3.2.3 MFC performance during phenanthrene degradation

A marked variation in electrochemical performances of different inocula (Figure 3.4) is an implication of differences in the electrochemical behaviour of each inoculum type. The bacterial consortia MCP gave the highest power density of 1.25 mWm^{-2} while similar power densities of 1.1 and 1.05 mWm^{-2} were recorded for CO and MCS respectively (cf. 0.37 mWm^{-2} for MC). Power densities from MFCs with simple organic substrate only (i.e. glucose or pyruvate) was three (3) times higher than treatments (data not shown). This is likely expected as high power densities could be as a result of the presence of readily oxidisable compounds (like glucose or pyruvate) and their non-toxic effect compared to MFCs with phenanthrene (i.e. treatment). As previously discussed, *P.aeruginosa* could be forced to produce more redox electron shuttling molecules (i.e. pyocyanin or phenazines) from its cells under anaerobic conditions and its positive interaction with electrochemically-active microbes present in the anaerobic sludge may have contributed to high power density observed for the bacterial consortia, MCP. Figures 3.4B and 3.4C indicate that from

a power density perspective, mutual interactions exist between CO, MCS and MCP, which perhaps, may have positively contributed to differences in electrochemical performances of different strains investigated. One possible explanation for this could be the transfer (e.g. via horizontal gene transfer) of "electrogenic activity" to non-electrochemically active or redundant strains (Bader et al., 2010; Pham et al., 2006).

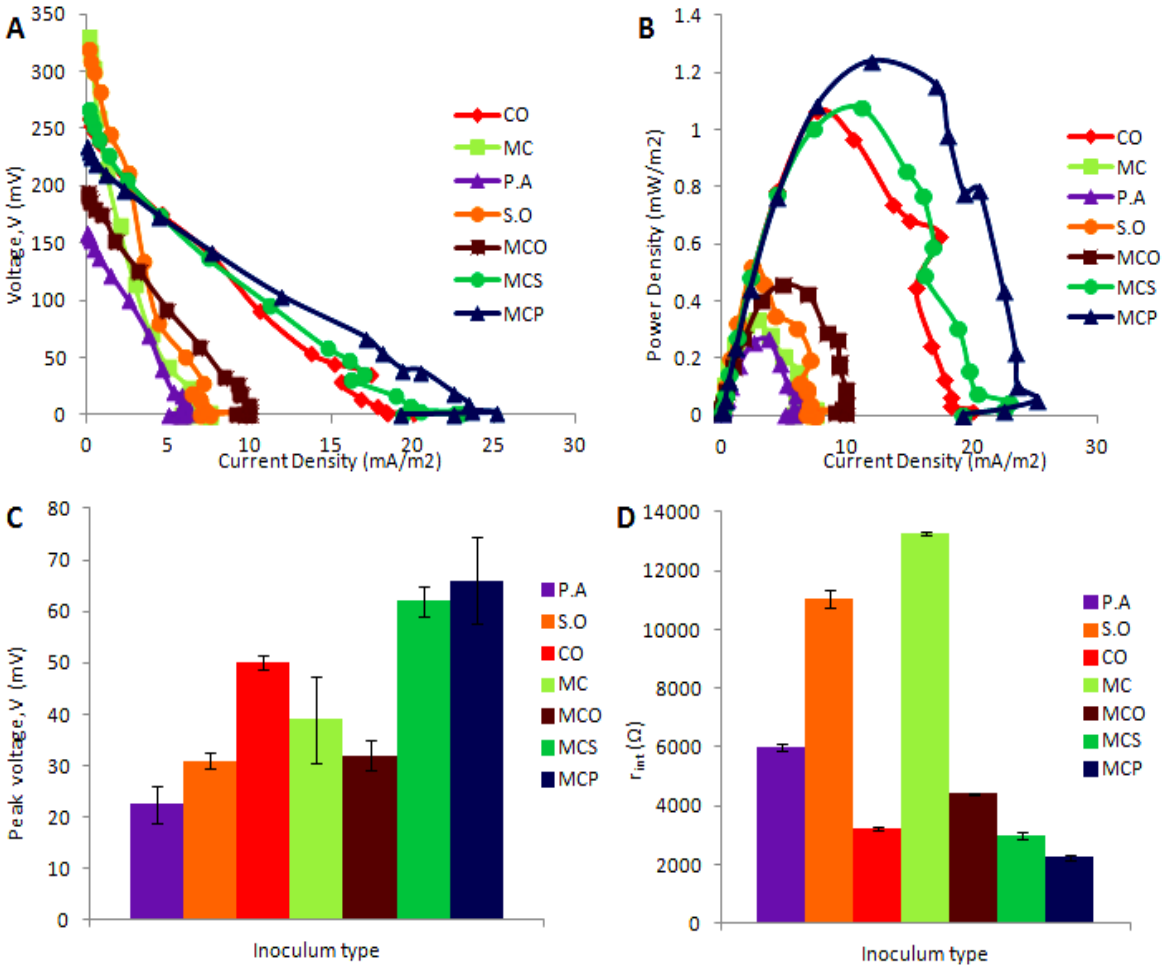


Figure 3.4: (A) Polarisation curves (B) Peak voltage outputs (C) Power density - current density plots (D) Internal resistances for degradation of phenanthrene for different inocula during MFC operation. The error bars represent the SD of the mean. *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS) and anaerobic digested sludge with *P. aeruginosa*, MCP.

In light of this, findings from this study suggest bioaugmentation could play an important role in enhancing electrochemical performances of the system in which it is being implemented.

Notably, there was a no correlation between power density and degradation rates (r = 0.07, $p<0.05$) (Figures 3.2B and 3.5); a similar observation was reported by Hu et al (2011). A number of factors e.g. oxygen intrusion into the anode, substrate conversion to biomass etc may confound the correlation between degradation rate and power density. The cell voltages and power densities for all inocula were inversely correlated with internal resistances of the cells (Figure 3.4) in accordance with Ohm's law.

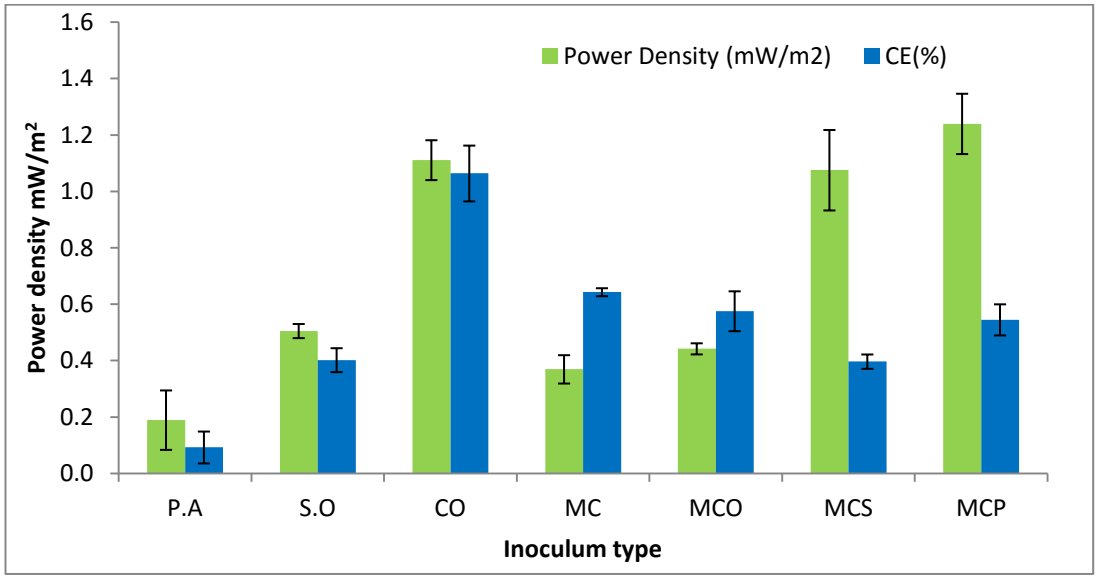


Figure 3.5: Comparison of power densities outputs and coulombic efficiencies for different inoculum types. Coulombic efficiency (CE), *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS) and anaerobic digested sludge with *P. aeruginosa*, MCP. Values are means of duplicate experiments ± SD.

Figure 3.5 shows that there is no correlation between power density and coulombic efficiency (r = 0.50, $p<0.05$). Coulombic efficiency recorded for all inocula tested

were generally low (<1.4 %), indicating a substantial loss of electrons within the system. The observed low coulombic efficiency may be due to oxygen diffusion into the anode chamber or a result of presence of alternative electron acceptors such as sulfates (0.25 g L^{-1} $MgSO_4$ was used in this study) which make up the anolyte medium (section 2.2). Adsorption of phenanthrene did not appear to interfere with electron transfer from bacterial cells to the electrode. Substrate oxidation by anodophiles with release of electrons occurs both in solution and on the anode.

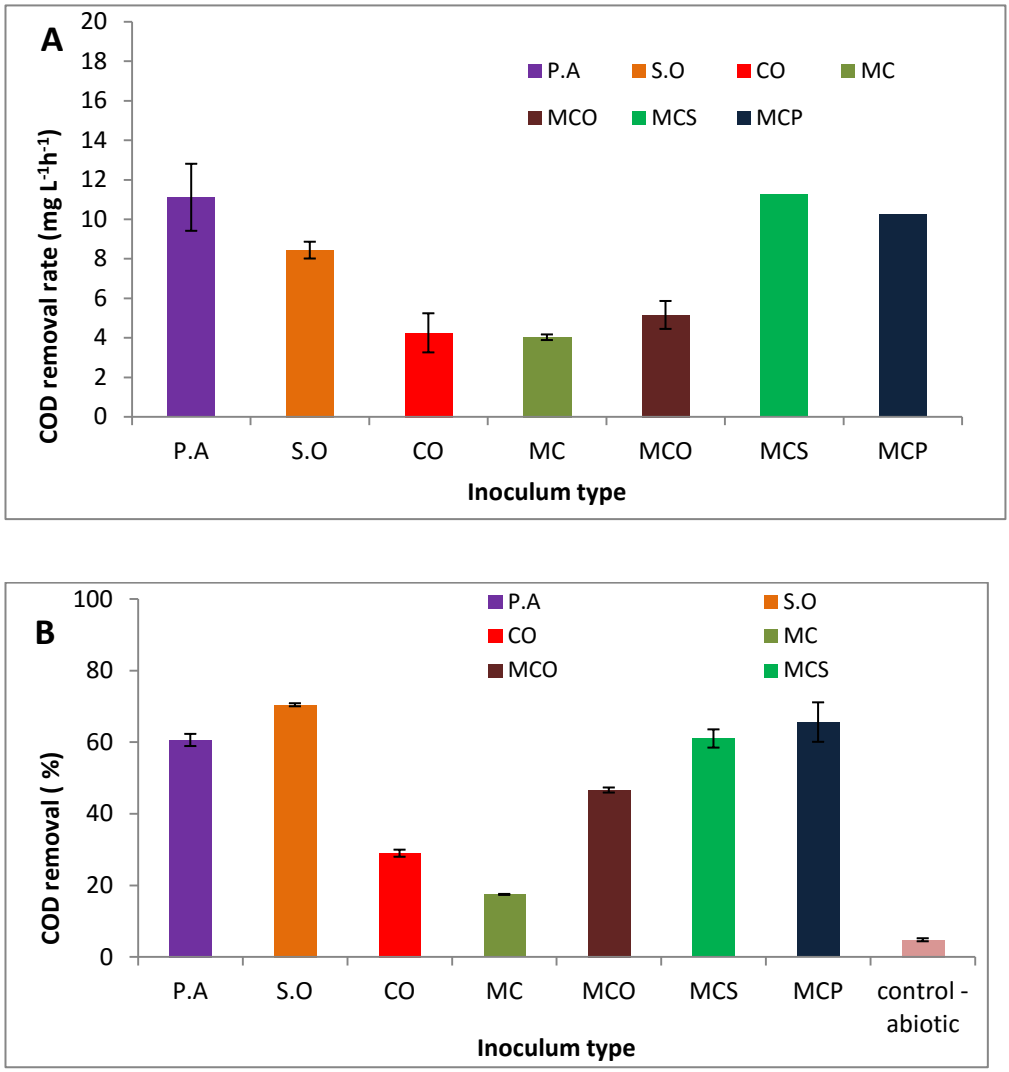


Figure 3.6: (A) COD % removal and (B) COD removal rate over the study period in a MFC for different strains. *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS) and anaerobic digested sludge with *P. aeruginosa*, MCP. Values are means of duplicate experiments ± SD.

Electrons released in the solution could be shuttled to the electrode by redox mediators secreted endogenously by the microorganism while electrons released on the anode can be transferred directly with the aid of c-type cytochromes (Liu et al., 2004b; Logan, 2008).

This study has demonstrated that closed circuit MFC systems were better than open circuit and anaerobic systems in terms of higher degradation rates and efficiency (Figures 3.2 and 3.3). This suggests that adsorption may not be detrimental to electron transfer.

However, the power densities and coulombic efficiencies, as previously discussed, do appear to indicate otherwise; but this can be explained by the large internal resistances measured in the MFC systems (Figure 3.4D). COD removal for different inocula during the MFC operation is shown in Figure 3.6. Cultures P.A, S.O, MCP and MCS gave COD removal percentages in excess of 50 % with COD removal rates above 8 mg L^{-1} h^{-1}. Notably, there was a large disparity between degradation efficiencies and COD removal efficiencies (Figures 3.2 and 3.6) though average degradation efficiencies across all inocula used in this study were above 90 %; however, this has not reflected in the COD removal efficiencies which are significantly lower (i.e. below 65 %) than degradation efficiencies. This disparity might be due to the presence of methanol which was used in dissolving the phenanthrene in the MFCs. The methanol used as a solvent for phenanthrene contributed significantly (about 75 % of the total COD values) for all inoculum types tested. Low coulombic efficiencies observed in this study (Figure 3.5) could also be attributed to low COD removal efficiencies. This suggests that the presence of methanol did not have any adverse impact on degradation of phenanthrene using different inoculum types in MFC systems. Findings from this study point to the

utility of *Pseudomonas aeruginosa* and *Shewanella oneidensis* as good candidates for MFC-based bioremediation as pure cultures or as supplements to mixed cultures.

3.2.4 Selection of the best performing strain(s)

The criterion for the assessment of MFC performance (using different inocula) was based on the degradation and electrochemical performances. Table 3.2 shows a summary of performances of different strains measured by a system performance index (**A**) as described in section 2.6.1.1. In considering the best inoculum for MFC systems, this order/degree of preference - max. power density > % COD removal > degradation rate, must be put into consideration in the assessment of system performance. In terms of power generation, MCP, MCS and CO gave the best performance as there were no statistically significant difference ($p<0.05$) in their power outputs. However, considering the second order of preference which is the % COD removal, SO was the best choice while MCP outwits MCS and CO significantly ($p<0.05$).

Table 3.2: Evaluation of system performances by different inocula based on three main parameters. Values are means of duplicate experiments ± SD.

Inoculum type	**Degradation rate, x ($mg\ L^{-1}\ d^{-1}$)**	**Max. power density, P_{max}, y (mWm^{-2})**	**COD removal, z (%)**	**System performance index, A= xyz/1000**
S.0	4.84±0.52	0.51±0.03	70.43±0.42	0.21
P.A	9.73±0.60	0.19±0.05	60.61±1.69	0.15
CO (P.A&S.O)	4.89±0.28	1.11±0.07	28.98±0.99	0.18
MC	6.35±0.27	0.37±0.05	17.47±0.14	0.05
MCO	6.50±0.60	0.44±0.02	46.63±0.71	0.16
MCS	4.88±0.20	1.05±0.14	61.02±2.55	0.38
MCP	4.93±0.11	1.25±0.05	65.60±5.52	0.49

In terms of degradation rates, PA showed the best performance compared to others followed by MC and MCO; there was no significant difference ($p<0.05$) in their degradation rates (i.e. MCO and MC). MCP, MCS and SO gave the least degradation performance and notably, there were no statistically significant difference ($p<0.05$) in their degradation rates.

Since the most important parameters in MFC systems are power generation and treatment efficiency (in terms of COD removal), coupled with the data from the K values, MCP is considered to be the best inoculum in the order of overall system performance. This suggests the use of MCP for bioaugmentation purposes in treatment of phenanthrene-contaminated sites based on MFC technology.

3.2.5 Benzene biodegradation during MFC operation

3.2.5.1 Effect of inoculum type on benzene degradation.

The degradation rates and removal of benzene during MFC operation are represented in Figure 3.7. All the eight inocula showed very high potential for benzene removal with minimum degradation efficiency of 86% compared to the controls (Figure 3.8). Notably, a temporary rise in benzene concentration in aqueous phase was observed few hours (*ca*. 10-12 h) after start up (Figure 3.7). The observed rise in benzene concentration, as apparent for all microbial consortia tested (but highly significant for the abiotic control), could be attributed to the fact that desorption rates of adsorbed benzene were higher than degradation rates in the course of the whole biodegradation process (Xia et al., 2010). Since no biotic degradation is occurring in the abiotic MFCs control, the residual benzene concentration in the aqueous phase remain higher than the biotic MFCs and was fairly stable throughout the experimental period. There was a statistically significant difference in one way

ANOVA ($p<0.05$) in the degradation performances of different inocula tested which suggests benzene degradation in MFCs is a function of inoculum type.

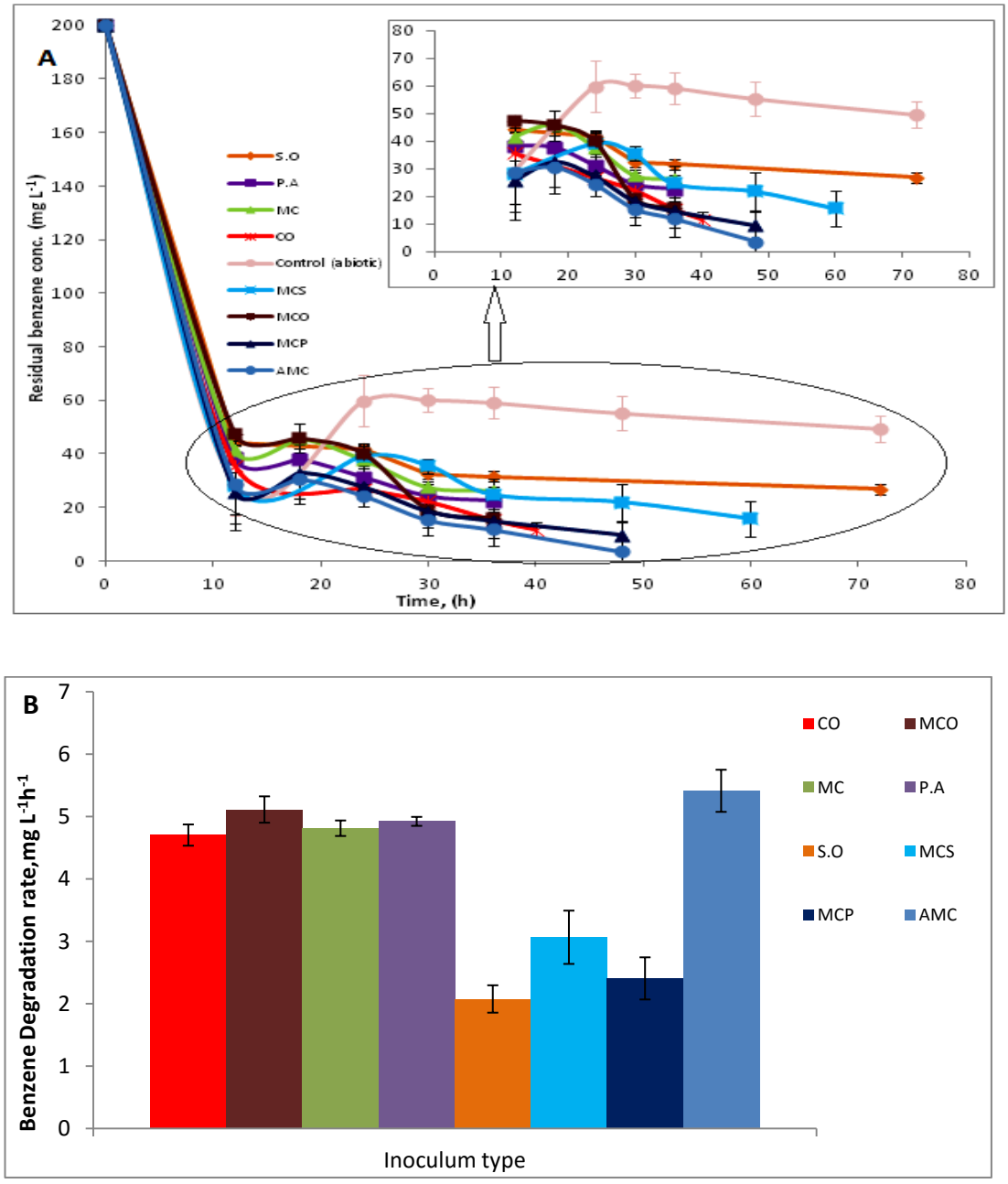


Figure 3.7: (A) Benzene concentration in the bulk anode solution as a function of time (and inset: A clearer picture of the plot of residual benzene concentration against incubation time between 10 and 90 h) and (B) benzene (average) degradation rates by different inocula used in this study. *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS), anaerobic digested sludge with *P. aeruginosa*, MCP and adapted mixed culture (AMC). The error bars represent the SD of the mean.

In this present study, AMC gave the highest degradation rate of 5.41 mg $L^{-1}h^{-1}$ with *S.oneidensis* giving the lowest degradation rate of 2.10 mg $L^{-1}h^{-1}$ as shown in Figure 3.7. This indicates that all the inocula tested may possess the aromatic hydrocarbon degrading enzymes (though at comparatively varying degree of enzymatic activity) which facilitated the degradation of benzene under anaerobic conditions. Notably, there is a statistically significant difference ($p<0.05$) among inoculum types except between MC, CO and PA. Enhanced oxidation of benzene was achieved with the anode serving as electron acceptor, hence making the metabolic process more favorable thermodynamically for the microorganism to probably meet their energy requirements for cell synthesis and maintenance.

Degradation rates recorded in this study are relatively higher than those reported in the literature where other alternative electron acceptors (such as nitrates, sulphates, etc.) were used to enhance anaerobic degradation of benzene in different contaminated environments (Dou et al., 2008; Musat and Widdle, 2008; Schreiber and Bahr, 2002). Cervantes et al (2011) reported degradation rate of 0.22 mg $L^{-1}h^{-1}$ for benzene anaerobic degradation using a humic model compound, anthraquinone-2, 6-disulfonate (AQDS), as terminal electron acceptor in a contaminated soil environment. In another study, Chakraborty et al (2005) demonstrated anaerobic degradation of benzene by *Dechloromonas* strain RCB using different TEAs. They reported degradation rate of 0.02 mg $L^{-1}h^{-1}$ (0.25 μMh^{-1}) which also far lower than that (using MFC system) reported in this study. This suggests a better degradation performance using MFCs (in this study) compared to other anaerobic conventional systems.

Benzene removal during MFC operation involves two processes namely; adsorption and microbial degradation. The first step is adsorption (which accounts for rapid

disappearance of benzene in aqueous phase) followed by microbial degradation. Rapid disappearance of benzene from the aqueous solution was observed as shown in Figure 3.7. The reason for this could be attributed to the adsorption of significant amount of benzene by the anode. As previously mentioned, the anode was made up of a carbon-felt paper which is a common type of carbon material widely used in MFC studies. Carbon materials (e.g. carbon-felt used as anode) have been reported previously to possess high adsorption capacity and this could be probably due to the nature of material and its specific surface area (Zhang et al., 2010a; Xia et al., 2010). On the other hand, the model hydrocarbon (benzene) used in this study is generally classified as a hydrophobic compound and therefore exhibits high affinity to solid materials (Xia et al., 2010; Velvizhi and Venkata Mohan, 2011). Nevertheless, electrochemically active microorganisms could still utilize adsorbed benzene on the anode. The findings reported in the present work is also in agreement with Zhang et al (2010a) who also demonstrated that adsorbed benzene on the electrode were subsequently metabolized by microorganisms.

Possible mechanisms for microbial metabolism of the adsorbed benzene could be mediated by the presence of anode-respiring bacteria or exoelectrogenic microorganisms. Benzene adsorption on the anode probably had no detrimental effect on electron transfer to the anode since degradation efficiencies were better in MFCs than non-MFCs (anaerobic control) as shown in Figure 3.8. Connected MFCs were also statistically significant ($p<0.05$), in terms of degradation efficiencies, than disconnected MFCs using different inoculum types (Figure 3.8). Benzene adsorption onto the electrode thus could enhance the removal efficiency in general, assuming the adsorbed benzene is being degraded as postulated in this study (Chandrasekhar and Venkata Mohan, 2012). Therefore, it is suggested that the presence of such

carbon-like electrode (acting as electron acceptors) could further enhance removal of petroleum hydrocarbons pollutants from contaminated site, especially in sub-surface environments where accessibility of microorganisms to contaminants poses one of the major challenges in the deployment of conventional remediation technologies.

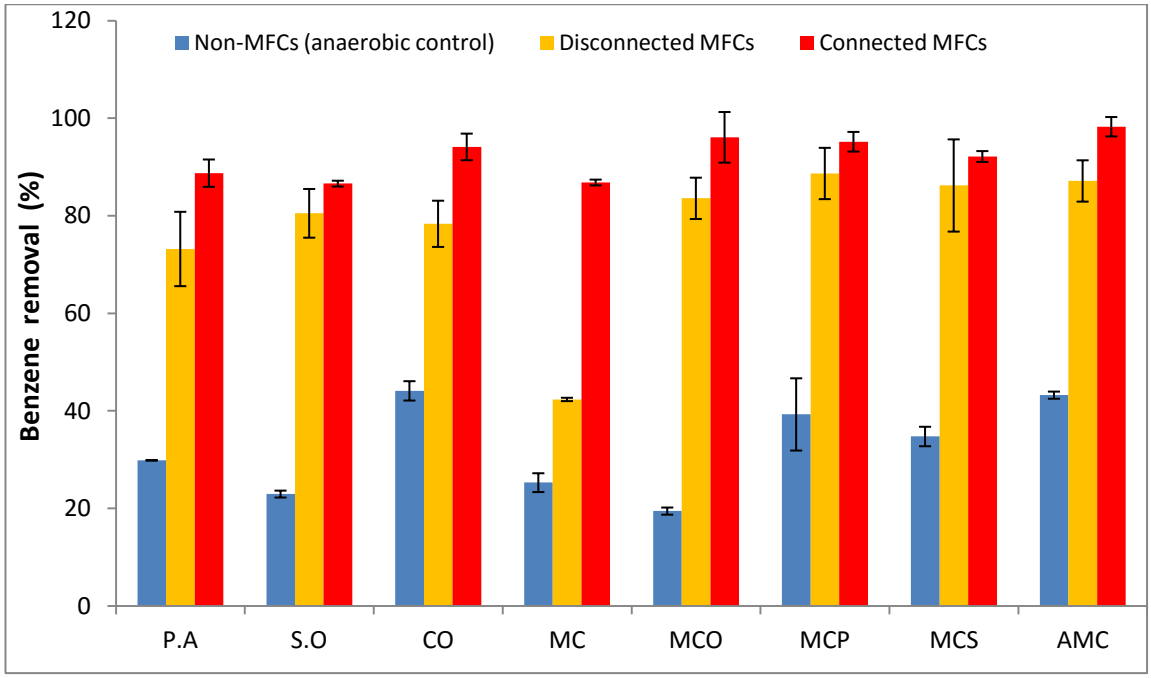


Figure 3.8: Degradation efficiencies for benzene removal by different inoculum types. *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS), anaerobic digested sludge with *P. aeruginosa*, MCP and adapted mixed culture (AMC). The error bars represent the SD of the mean.

Anaerobic degradation of benzene in MFC generally involves extracellular transfer of electrons resulting from microbial oxidation of the substrate to an anode. Extracellular electron transfer by anode respiring bacteria could be either by direct use of pilus-like assemblages known as *nanowires*, which have been reported found in *Geobacter spp* and *S. oneidensis* or electron transfer mediated by the use of soluble redox electron shuttles - indirect electron transfer (Rozendal et al., 2008; Hawkes et al., 2010; Logan, 2008). *P.aeruginosa* and *S. oneidensis* have been widely

reported in the literature to secrete pyocyanin and flavin-like molecules respectively (Rabaey et al., 2005). Adapted-anaerobic mixed cultures also have been reported to contain diverse anodophilic microorganisms that could mediate electrons transfer vis-a-vis substrate oxidation.

Both mixed culture and axenic culture that possess benzene degrading enzymes have been reported extensively in the literature (Brusa et al., 2001; Cervantes et al., 2011; Dou et al., 2008; Meckenstock et al., 2005). Pure strains of *Pseudomonas species* have been reported by some authors to have a potential to degrade aromatic hydrocarbons – including BTEX compounds (Brusa et al., 2001; Ridgeway et al., 1990; Sahar et al., 2010). *S.oneidensis* MR1 used in this study may also possess benzene degrading enzymes that enabled biodegradation of the hydrocarbon pollutant under anaerobic conditions.

To the best of authors' knowledge, this is the very first report that demonstrated that pure strains such as *P.aeruginosa* and *S.oneidensis* can oxidise benzene in a microbial fuel cell. In a study conducted by Morris et al (2009) on the enhanced anaerobic degradation of diesel using MFCs, *S.oneidensis* was found among electrochemically active bacteria present in the anode of the MFCs as revealed by phylogenetic analysis. Similarly, Cervantes et al (2011) reportedly found *Shewanella spp.* among facultative anaerobes present in benzene contaminated sediments. Thus, this suggests *S.oneidensis* could, in principle, potentially degrade petroleum hydrocarbons coupled with concomitant electricity generation via the anode. This proposition has been supported by findings reported in this study.

The mechanism of anaerobic degradation of benzene is unclear. Anaerobic biodegradation of benzene has been reported previously to undergo several possible

mechanisms such as benzene methylation, carboxylation, alkylation, reduction, or hydroxylation reactions followed by ring cleavage. Whichever the case, they are all channeled through a central intermediate metabolic pathway called the benzoate metabolic pathway in the presence of benzoyl-CoA enzymes (Coates et al., 2002). In this study, some acidic intermediates were formed as indicated by a decrease in measured pH (from 7.0 to about 5.6) during benzene degradation for all inocula tested. However, identification of these degradation products with the hope of identifying metabolic pathway for benzene degradation is difficult owing to the presence of other carbon sources.

Bioaugmentation is one of the bioremediation techniques employed in the enhanced biodegradation of contaminated sites where natural attenuation are too slow and proves ineffective (Thompson et al., 2005). However, the success of these techniques relies solely on the adaptability and microbial interactions among the bioaugmented species to the environmental conditions that exists in the site of application. Moreover, in this study, all the eight inocula gave different degradation rates and efficiencies suggesting the existence of microbial interactions among bacterial species employed in this study as presented in Figures 3.7 and 3.8. Among the range of inocula tested, the bacteria consortia-AMC shows the best degradation performance. Therefore, findings for this study suggest AMC as a possible choice of strain for bioremediation of benzene in contaminated environments.

3.2.6 Electrochemical performances of different inoculum type in MFCs

As shown in Figure 3.9, there was a marked variation in the electrochemical performances exhibited by different inocula which is a function of inoculum type. The bacterial consortium - AMC gave the highest power density of 0.82 mWm^{-2} which was 40-fold compared to MC (0.02 mWm^{-2}). In terms of maximum power

densities, there was no significant difference among SO, PA and MCO based on ANOVA tests ($p>0.05$). High power density recorded for AMC might be due to the combined effect of presence of both electrogenic and benzene-degrading bacteria which could have enhanced MFC performance. Second to AMC in terms of power density was CO which showed a higher electrochemical performance than other inoculum types.

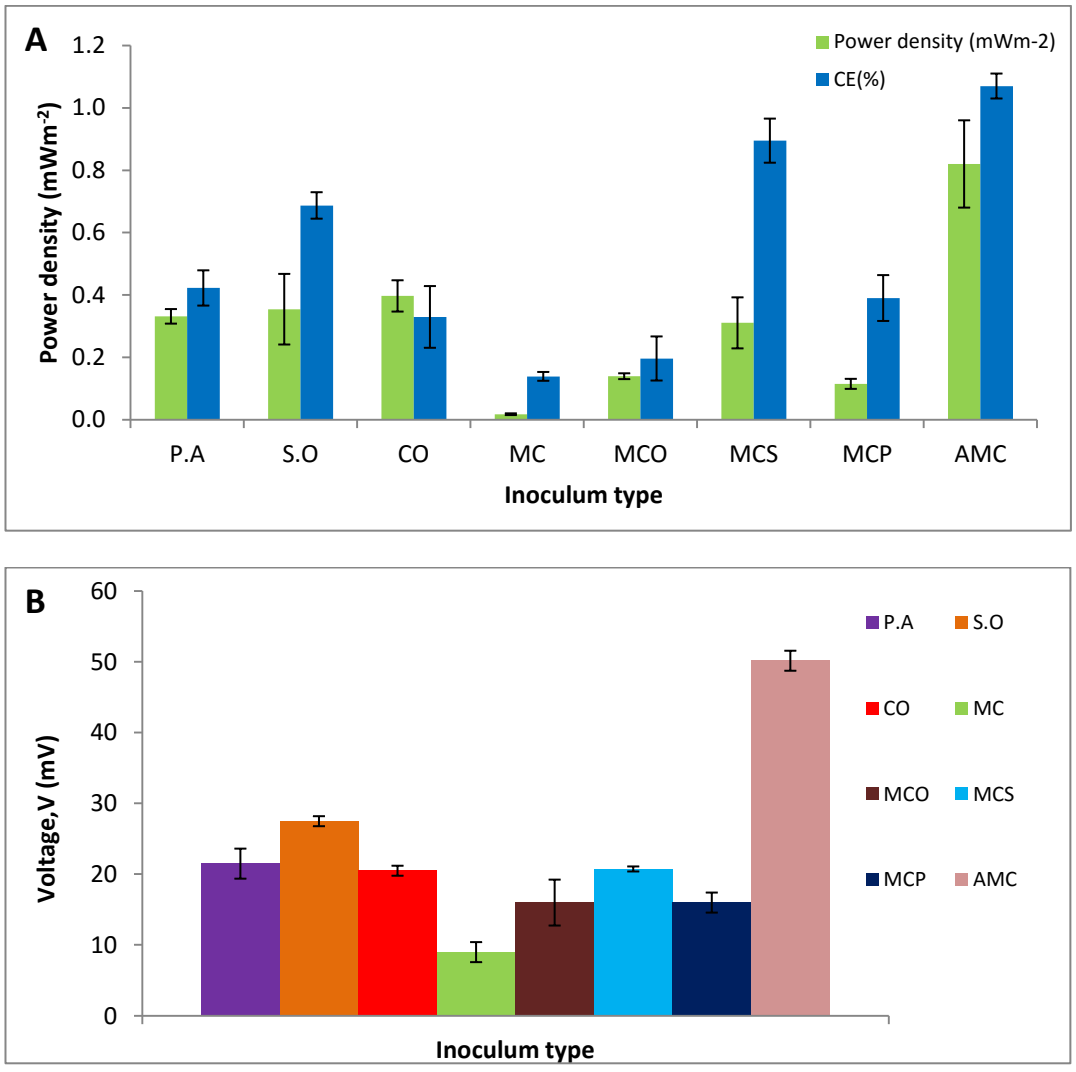


Figure 3.9: (A) Maximum power densities, coulombic efficiencies and (B) Peak voltage outputs for degradation of benzene for different inocula during MFC operation. The error bars represent the SD of the mean. *S. oneidensis* (S.O), *P. aeruginosa* (P.A), a co-culture of *S. oneidensis* and *P. aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the co-culture (MCO), anaerobic digested sludge with *S. oneidensis* (MCS), anaerobic digested sludge with *P. aeruginosa*, MCP and adapted mixed culture (AMC).

This reason for CO's performance might be due to synergistic interactions that existed between PA and SO. PA and SO have been reported to be electrochemically active microorganism (Logan, 2008; Hawkes et al., 2010). PA excretes redox molecules such as phenazines and pycocyanin which act as electron shuttling agents by transporting electrons across the cell membrane to the anode while SO produces flavin-like molecules.

Increased concentrations of such redox mediators could have facilitated high electron transfer rates which could have resulted in high the peak power density produced in this study for the co-culture (CO). Interestingly, a good electrochemical performance might not necessarily translate into better degradation efficiency or rate under certain conditions. In this study, we observed that there was disparity between biodegradation rates and power generation for different inocula tested in the MFCs (Figures 3.2B and 3.9A). Many factors could be responsible for this; one of the critical factors influencing system performance could be electrochemical capability of the microorganism.

Hu et al (2011) reported similar observations where the relationship between power density and degradation rates using different substrates was investigated. Coulombic efficiency recorded for all inocula tested were generally low (within 0.14-1.04 %) (Figure 3.9A). The observed low coulombic efficiency may be due to ingression of oxygen into the anode, soluble substrate loss due its diffusion into the cathode (in case of dual MFCs), incomplete substrate oxidation or presence of other alternative electron acceptors such as sulfates (Morris et al., 2009; Kim et al., 2007). The nature of microorganisms or their consortia (either pure or mixed culture) may also

contribute to the low efficiency observed in the study. The presence of non-exoelectrogenic species such as fermentative bacteria and methanogens (e.g. in mixed consortia) could be a limiting factor to coulombic efficiency yield (Rodrigo et al., 2010).

3.2.7 Assessment of MFC performance for benzene degradation

In this study, MFC performances of all inocula were assessed based on their degradation and electrochemical performances using a system performance index, **K** as described in section 2.6.1.3. In considering the best inoculum for MFC systems, this order of preference - max. power density > % COD removal > degradation rate, must be put into consideration in the assessment of system performance. In terms of degradation rates, AMC showed the best performance compared to others followed by MCO; there was no significant difference ($p<0.05$) in the degradation rates for PA, MC and CO. AMC also showed very good performance In terms of power generation with MC being the least in the order of performance. However, for the % COD removal, AMC was the best choice followed by MCO which showed better performance than SO, PA and CO significantly ($p<0.05$). There were, however, no statistically significant difference ($p<0.05$) in the degradation rates for SO and PA. Based on the performance parameters, bacterial consortium - AMC was found to be the best performing inoculum with MC being the least in order of overall system performance (Table 3.3). Findings from this study recommend the use of AMC for bioaugmentation purposes in the remediation of benzene-contaminated sites based on MFC technology.

Table 3.3: Evaluation of system performances by different inocula based on three main parameters.

Inoculum type	Degradation rate, x (mg $L^{-1}h^{-1}$)	Max. power density, P_{max}, y (mWm^{-2})	COD removal, z (%)	System performance index, K = xyz/1000
S.0	2.08±0.22	0.33±0.02	63.05±0.42	0.05
P.A	4.92±0.07	0.35±0.01	63.38±3.69	0.12
CO(P.A&S.O)	4.70±0.17	0.39±0.05	61.76±1.56	0.14
MC	4.81±0.12	0.02±0.00	66.67±7.07	0.01
MCO	5.11±0.21	0.14±0.02	70.00±1.69	0.06
MCS	3.07±0.42	0.31±0.08	26.32±0.71	0.04
MCP	3.96±0.33	0.12±0.01	50.00±0.74	0.03
AMC	5.41±0.62	0.82±0.14	87.25±2.21	0.52

3.2.8 Toxicity reduction during MFC operation

Microbial degradation of pollutants often results in incomplete mineralization and hence the formation of degradation products with unknown chemical and toxicological characteristics. In this study, toxicity assays were conducted for both pre-treated and treated liquid samples (in MFC seeded with adapted mixed culture as inoculum) at 200 mg L^{-1} benzene concentration (Figure 3.10). Result indicates that degradation products are less toxic than the parent pollutant at the benzene concentration tested. The half maximal luminescence inhibition value (EC_{50}) for the treated and pre-treated solutions were 149.4 mgCOD L^{-1} and 50.3 mgCOD L^{-1} respectively using bioluminescence toxicity assay (section 2.7.6). The EC_{50} values for treated sample was three-fold higher than the pre-treated sample suggesting a statistically significant ($p< 0.05$) lower cytotoxicity effect based on bioluminescent organism used (*V.Fischeri*).

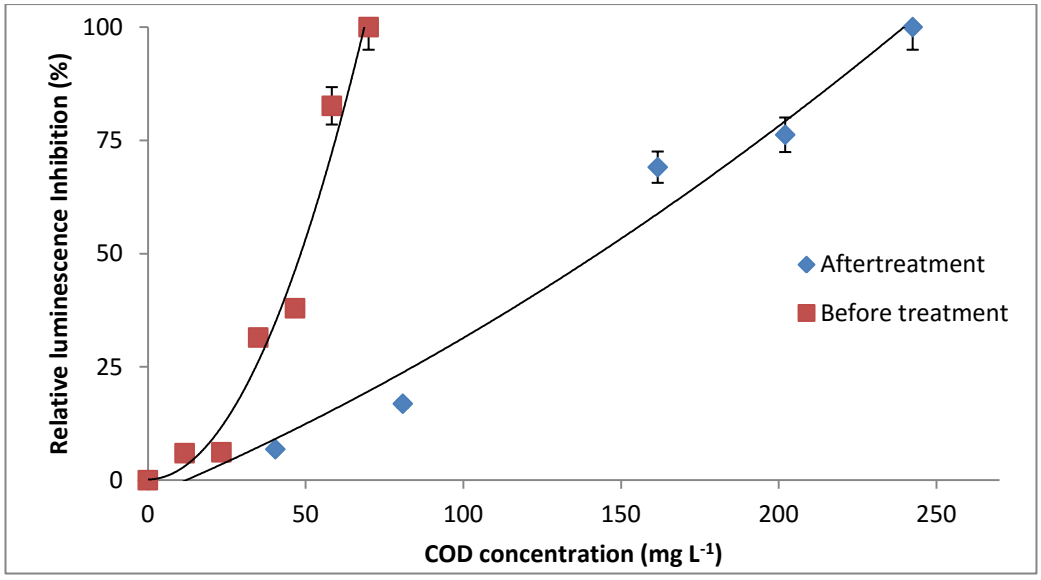


Figure 3.10: *Vibrio fischeri* bioluminescence based toxicity determinations of pre-treated and treated samples from MFC seeded with MFC-adapted mixed culture (at 200 mg L^{-1} benzene concentration). Values are means of duplicate experiments ± SD.

Increase in EC_{50} values is an indication of decreasing toxicity compared relatively with the pre-treatment samples. Result suggests the possible formation of degradation products mixtures of lower molecular weight compounds like volatile fatty acids or other simpler acid metabolites which have may have lower toxic effects than the parent pollutant - benzene (Cooper et al., 2010). Proof of some acid metabolites present in samples was further supported by decrease in pH of samples compared to pre-treatment samples (i.e. from pH 7 to 5.6). The findings of this study further reinforces the evidence reported in the previous sections about the effective degradation of benzene-contaminated synthetic wastewater using MFC as it is the ultimate goal of any remediation strategy is to convert toxic contaminants into less toxic or non-toxic forms (Xiong et al., 2012).

3.2.9 Effect of organic substrate concentration on MFC performance.

The influence of organic substrate concentration on MFC performances, using two different inocula (*S.oneidensis* MR1 and MFC-adapted mixed culture (section2.6.1.4)), was evaluated. As shown in Figure 3.11, there was a gradual

decrease in peak voltage outputs with sequential decline in organic substrate concentrations. Changes in organic substrate's concentrations did not cause any significant difference in coulombic efficiencies produced by the MFC system (Table 3.4) using MFC-adapted mixed culture (section 2.6.1.4). Similiarly, the same trend was observed with the pure culture (using pyruvate (50 mg L^{-1}) as organic substrate.

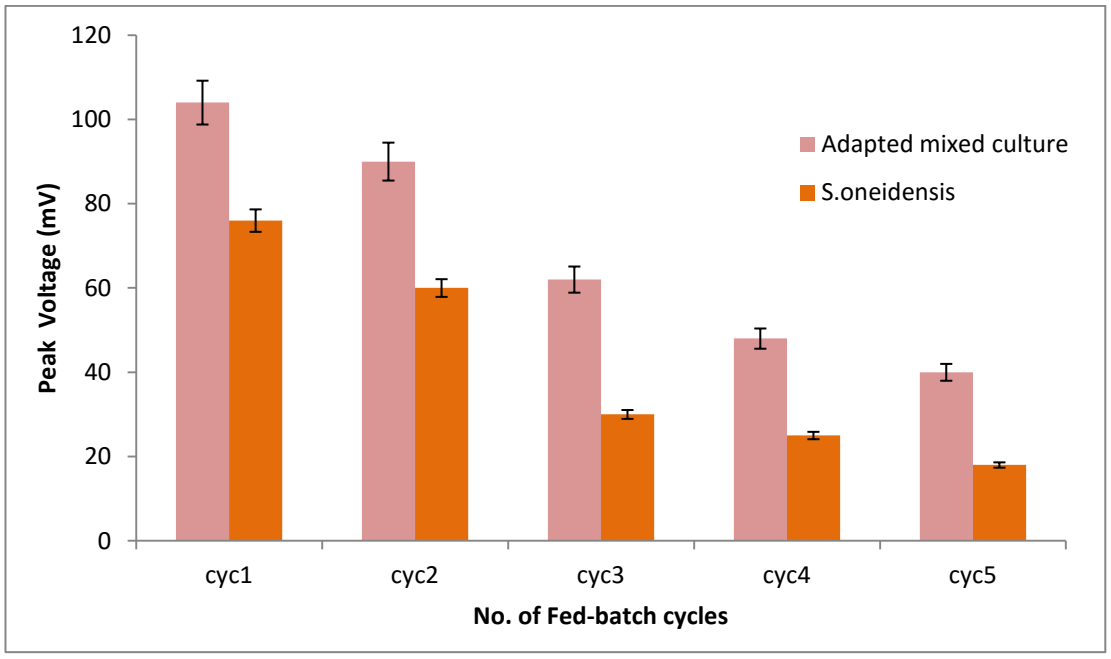


Figure 3.11: Comparison of peak voltages output of the two inoculum types (*S.oneidensis* MR1 and MFC-adapted mixed culture). Values are means of duplicate experiments ± SD.

Table 3.4: Coulombic efficiencies at varying organic substrate concentrations for different inoculum types. Values are means of duplicate experiments ± SD.

Inoculum type	***S.oneidensis***		**MFC-adapted mixed culture**	
Organic substrate (mg L^{-1})	**Sodium Pyruvate**	**CE (%)**	**Glucose**	**CE (%)**
	2200	0.32±0.06	1000	0.45±0.01
	550	0.35±0.02	400	1.04±0.11
	200	0.11±0.01	200	0.59±0.07
	100	0.32±0.02	100	0.53±0.03
	50	0.33±0.01	50	0.73±0.09

From Figure 3.12 as observed, the stepwise decrease in the amount of organic substrate had no statistically significant effect (p<0.05) on the degradation efficiencies of the petroleum hydrocarbons mixture for both inoculum types. Results indicate that about 71 % COD removal was achieved when 100 mg L^{-1} glucose was used as organic substrate for the degradation of the selected hydrocarbons using a mixed culture though voltage output was low (peaked at 45 mV) probably due to toxicity effect of contaminants (mixture of benzene and phenanthrene) on the inoculum. Similiarly, same trend was observed with the pure culture (using pyruvate (50 mg L^{-1}) as organic substrate.

This result suggests the selection of 100 mg L^{-1} glucose and 100 mg L^{-1} pyruvate as organic substrates for the mixed culture and the pure culture respectively in subsequent studies. The findings from this study recommend the use of lower amounts of organic substrate in field applications with a view of reducing remediation costs while maintaining optimal remediation efficiency (Zhang et al., 2010a).

Notably, there was a significant divergence between hydrocarbon and COD removal efficiencies (Figure 3.12). Degradation efficiencies (above 90 %) using different inoculum types was significantly higher than COD removal efficiencies (which ranged between 40 % and 75 %). This disparity might be due to the incomplete degradation or biotransformation of phenanthrene, benzene, formation of acidic metabolites (resulting from partial utilisation of glucose or pyruvate) and the presence of methanol which was used in dissolving the phenanthrene in the MFCs.. Low coulombic efficiencies observed in this study (Table 3.4) could also be attributed to low COD removal efficiencies.

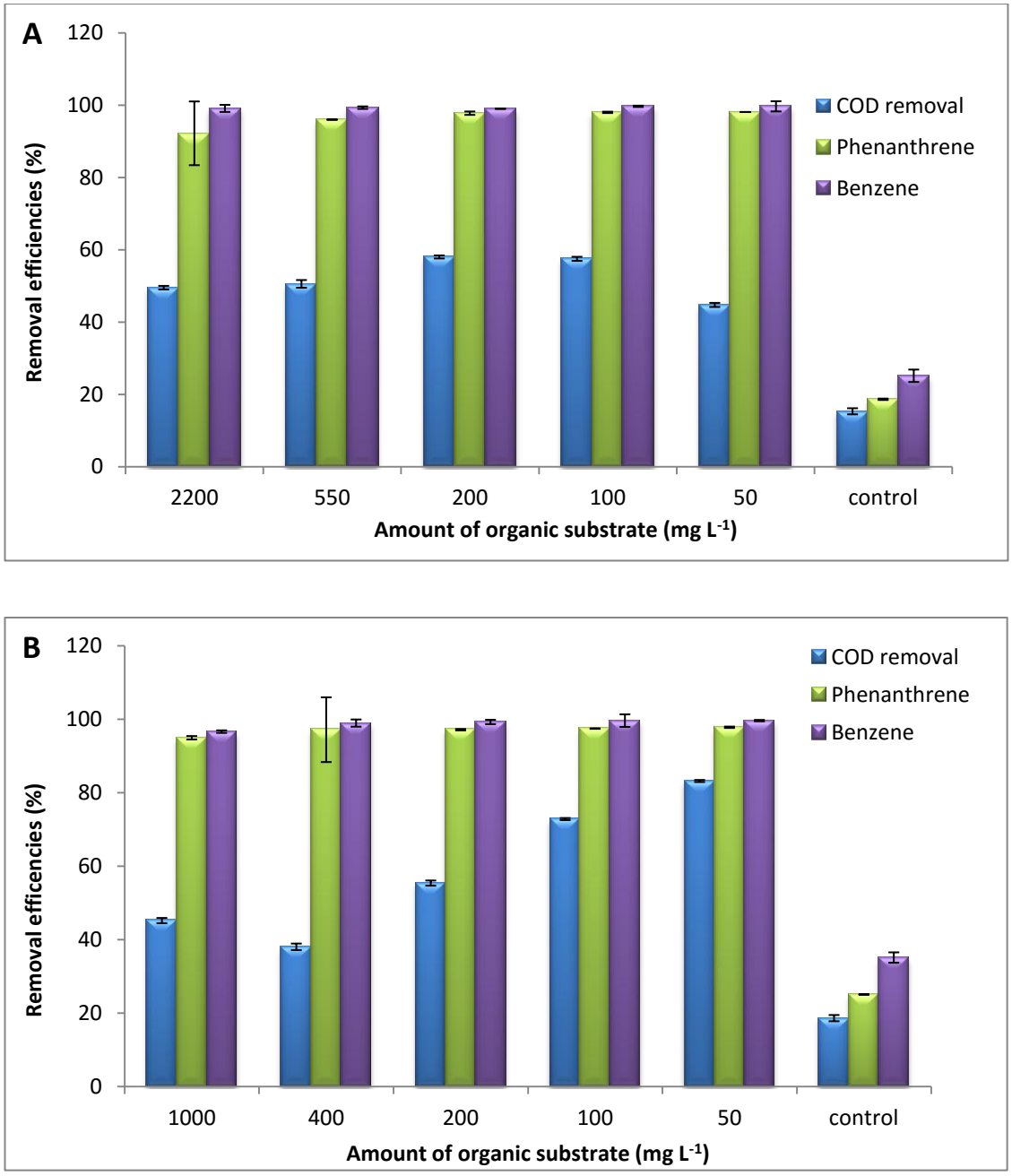


Figure 3.12: Removal efficiencies for degradation of phenanthrene and benzene using (A) *S.oneidensis* MR1 and (B) MFC-adapted mixed culture as inoculum respectively. Values are means of duplicate experiments ± SD.

Notably, there was a linear relationship with current outputs and organic substrate concentrations ranging from 50 to 550 mg L^{-1} only (Figure 3.13). This linear response observed suggests the possible use of MFCs for indirect determination of the toxicity of the target substrate.

Toxicity of petroleum hydrocarbons can inhibit microbial activity at the anode. In this study, a stepwise decrease in COD removal efficiency was observed as organic substrate concentrations were reduced gradually. This observed trend was also consistent with decrease in peak voltage outputs by MFCs as shown in Figure 3.11.

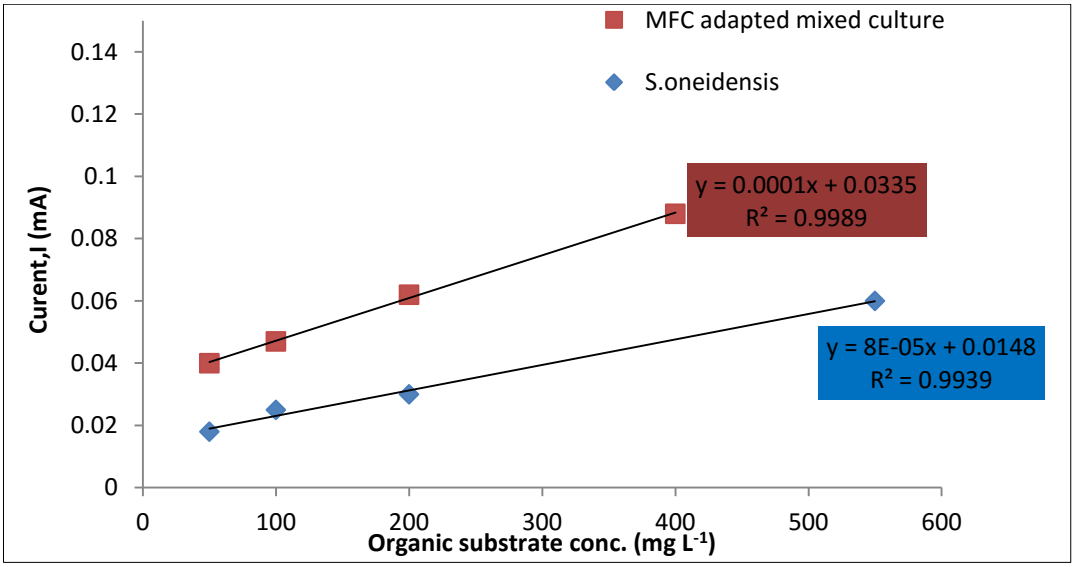


Figure 3.13: Relationship between organic substrate concentration and current generation from MFCs for different inocula (*S.oneidensis* and MFC-adapted mixed culture).

Results indicate that decrease in COD removal efficiencies and voltage outputs could be due to the cytotoxicity effect of the petroleum hydrocarbon on physiology of the microbial population present in the anode of the MFCs. Toxicity is one of the critical factors that determines the ease of microbial biodegradation of recalcitrant petroleum hydrocarbons (Yuan et al., 2000). Petroleum hydrocarbons such as phenanthrene and benzene have been reported widely in the literature to pose cytotoxic and genotoxic effect on microorganisms (McConkey et al., 1997; Jang et al., 2007). This observation corroborates well with the result a similar study by Kim et al (2003) that reported a linear relationship between the maximum electrical currents produced and the COD concentration of the substrate (up to 300 mg L^{-1}). Findings from this study could also suggest the use of MFCs as a toxicity biosensor in the determination of

the presence of toxic substances in contaminated sites or in an event of the introduction of a toxic compound in a previously non-toxic environment as toxicity, which can inhibit microbial activity, could consequentially affect power outputs from MFC adversely.

3.3 Concluding remarks

The first part of this study demonstrated the possibility of using MFCs, utilising a range of inocula, to enhance the biodegradation of phenanthrene through with concomitant, albeit meagre electricity production. The best overall performing inoculum was MCP, a mixed culture supplemented with *P. aeruginosa*. The culture gave a phenanthrene degradation rate of 4.93 mg $L^{-1}d^{-1}$, a maximum power density of 1.25 mWm^{-2} and a COD removal of 65.6 %. Adsorption studies showed that phenanthrene exhibits a strong affinity for the carbon felt anode electrode conforming to a Type II isotherm and having a monolayer capacity of 0.088 mg/cm^2. It is suggested that *Pseudomonas aeruginosa* NCTC 10663 may offer good prospects for bioaugmentation of mixed cultures in MFCs for bioremediation of hydrocarbons.

For the second part of this study, the feasibility of using MFC technology in the degradation of a model BTEX compound (benzene) with concomitant electricity production in the presence of different inoculum types as microbial catalyst was investigated. The bacteria consortia- AMC was the best performing inocula. The culture gave a benzene degradation rate, maximum power density and COD removal of 5.41 mg $L^{-1}h^{-1}$, 0.82 mWm^{-2} and 87.3 % respectively. Hence, these findings suggest its potential use for bioaugmentation purposes in MFCs. The EC_{50} values, based on *Vibrio fischeri* ecotoxicity testing, for post treatment is three-fold higher than the pre-treatment suggesting a lower cytotoxicity effect. This work further

demonstrated that organic substrate's concentration have no significant impact on degradation performance. Thus recommending the use of lower amount of organic substrates in commercial application; thereby reducing treatment costs drastically. This work highlights the possibility of using MFCs to achieve high degradation rates of phenanthrene and benzene and could potentially be used as a replacement of permeable reactive barriers for remediation of hydrocarbon-contaminated groundwater or wastewater.

CHAPTER 4

Studies on the robustness of MFC systems: Effect of different operating conditions and the interactive effects among some selected parameters.

4.1 Chapter overview

To test for the operational envelope of microbial fuel cells when treating recalcitrant pollutants, it is necessary to investigate the response of the system under various levels of operating conditions e.g. salinity and temperature, external resistance, redox mediator and surfactant type among others. The few workers (Morris et al., 2009; Morris and Jin, 2012; Huang et al., 2011; Wang et al., 2012b) that investigated the treatment of petroleum hydrocarbons in MFCs did not consider the impact of variations in environmental conditions. Morris et al (2009) investigated the biodegradation of diesel in a dual MFC at 30℃; they achieved 82 % DRO (diesel range organics) removal over 21 days compared to an anaerobic incubated control, which achieved 31 % removal. Chandrasekhar and Venkata Mohan (2012) investigated the effect of substrate concentration on degradation of real field petroleum sludge with concomitant bioelectricity generation by varying the organic loads operated at ambient temperature (29℃). Highest total petroleum hydrocarbons (TPH) removal of 35 % was obtained at lower organic loads (0.76 g L^{-1} TPH).

Knowledge of the operational envelope of MFCs to different operating conditions is important in assessing the technical feasibility of these systems. A change in operating temperature could adversely affect the nature and the distribution of the microbial population present in the anodic chambers of an MFC (Larrosa-Guerrero et al., 2010; Michie et al., 2011). Low temperatures may inhibit the growth of methanogenic bacteria but favour electrogenesis. An increase in temperature may increase biokinetics (mass transfer coefficient, activation energy, etc.), the system's thermodynamics and rate of substrate utilization (Larrosa-Guerrero et al., 2010; Boghani et al., 2013).

The presence of a redox mediator is one of the key mechanisms that has been suggested for the transfer of electrons from the electrochemically active bacteria to the anode. The use of exogenous redox mediators could enhance electrochemical performance of an MFC system by improving electron transfer rates (Keck et al., 2002). The key factors to consider are the redox potential of the mediator in relation to that of substrate oxidation and the anode potential, the permeability of the cell membrane for the redox mediator molecules and the toxicity of the redox mediator. Addition of sodium chloride to increase salinity could increase the conductivity of the anodic solution and may also decrease the internal resistance within the MFC (Lefebvre et al., 2012b). Increasing conductivity may enhance MFC performance in terms of electricity generation by promoting the movement of ions quickly through the MFC system with less internal resistance. Salinity may induce contradictory effects e.g. while increasing conductivity of the anolyte, the physiology of the microbial population present in the anode maybe adversely affected (Minai-Tehrani et al., 2009).

In field applications, one or more influencing factors (interacting with one another) that could affect system performance are often encountered (Mohajeri et al., 2010). Conventional methods of studying a process by keeping other factors constant does not depict the combined effects of all the factors involved. In order to evaluate and optimize BES performance, a well-known statistical tool, response surface methodology (RSM) can be employed. It has been widely used for optimisation of different system/process performances (Mohajeri et al., 2010; Zhang et al., 2012b). Parameters of significant interest used in this present study are salinity, external

resistance and redox mediator. The choice and levels of these parameters were based on preliminary studies reported in this chapter.

Therefore the purpose of the studies reported in this chapter was to investigate the performance of MFCs subjected to different operating or treatment conditions. RSM was performed to evaluate interaction between selected operating conditions and optimize the MFC system, with the target of maximizing power outputs. A semi-continuous MFC operation was also conducted to test the stability in the systems overall performance over long periods (60 days). The performance of the system was evaluated in terms of degradation performance (substrate's degradation rate and COD removal efficiency) and electrochemical performance (i.e. voltage outputs, internal resistance, maximum power generation and coulombic efficiency (CE).

4.2 Results and discussion

4.2.1 Stability of MFC performance during fed-batch MFC operation

4.2.1.1 Degradation of petroleum hydrocarbons during prolonged fed-batch MFC operation.

The degradation performance of the hydrocarbon mixture in a MFC operated in a fed-batch mode was assessed over six cycles in terms of degradation efficiency and COD removal efficiency of the individual hydrocarbons (i.e. phenanthrene and benzene). The degradation efficiency of hydrocarbon mixtures and COD removal reached over 95 % and 80 % respectively in all of the cycles as shown in Figure 4.1. There was a statistically significant difference ($p<0.05$) between the treatment (connected biotic MFC) and the MFC abiotic control over the six cycles. Good reproducibility and removal efficiency of the hydrocarbon mixture using an MFC system was obtained when adapted anaerobic microbial consortia (section 2.3) was

employed in fed-batch operation. High degradation rates and removal efficiency observed both in this study and our previous studies could be attributed to the presence of aromatic degrading enzymes present in the adapted microbial consortia and the availability of an insoluble electron acceptor present in the anodic chamber. The presence of these enzymes could have facilitated increased cell metabolic rate that might have resulted into higher substrate utilization. The continuous availability of the anode (serving as an inexhaustible electron acceptor) for microbial respiration coupled with substrate degradation over six (6) cycles give credence to the selection of MFC system as a sustainable and affordable bioremediation technology for groundwater treatment over the maintenance cost of supplying alternative electron acceptors such as nitrates, sulphates or metallic oxides into such environments.

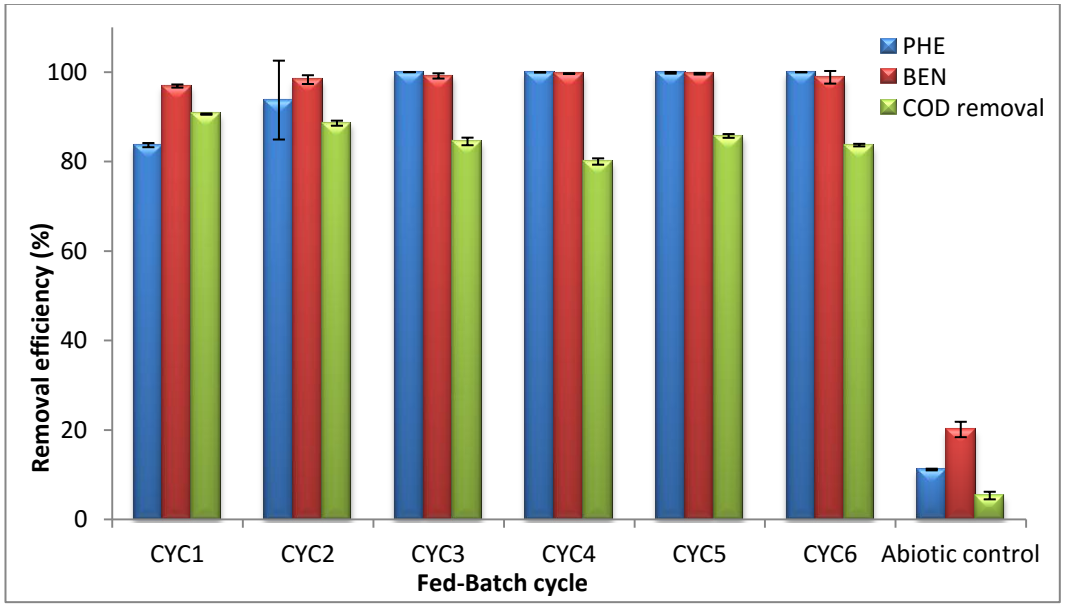


Figure 4.1: Percentage COD removal and degradation efficiencies of petroleum hydrocarbons mixture during MFC fed-batch operation using adapted microbial consortia. Values are means of duplicate experiments ± SD. Data for abiotic control represent the average values over six cycles.

Oxidation of substrates by anaerobic microorganisms via different metabolic pathways (depending on the nature of the substrate) has been well documented in the

literature (Meckenstock et al., 2005; Foght, 2008; Coates et al., 2002). Generally, anaerobic degradation of aromatic hydrocarbons proceeds in two steps; carboxylation of the aromatic ring followed by ring fission. The degradation pathway progresses until it enters into a central intermediate (i.e. the benzoate) metabolic pathway before further oxidation into CO_2 finally (Coates et al., 2002). During the degradation process, intermediates are formed, resulting from the biotransformation of the parent compound. However, the hydrocarbon mixtures used in this study further complicate the elucidation and characterization of the metabolites. Thus mapping out the degradation pathway in such mixed substrates would be technically difficult.

Biodegradation of hydrocarbon mixtures by microorganisms could be affected by substrate interactions such as competitive inhibition (Mathur and Majumder, 2010). Therefore, in the current study, we have considered two hydrocarbons of different homologues and investigated this inhibition effect. Degradation efficiency of about 99 % was achieved in cycles 3-6 when the MFC system was fully stabilized (Figure 4.1). This suggests that the effect of two different substrates interactions on microbial uptake was not inhibitory but rather promotes simultaneous degradation of both substrates. As previously discussed, this reason for the positive effect could be underpinned by the fact that the adapted anaerobic consortia possess versatile aromatic degrading enzymes that are capable of metabolizing both substrates. Degradation studies of hydrocarbon mixtures involving different homologues (i.e. PAH and BTEX compounds) have rarely been reported in the literature (especially in MFC systems) even though few studies have reported the effect of competitive inhibition on degradation of substrate mixtures. In one study, Lee et al (2002)

quantified the competitive inhibition kinetics of BTEX mixtures using *S. maltophilia* T3-c. They found that the presence of toluene or xylene in binary mixtures with benzene increased the specific degradation of benzene while benzene degradation was inhibited in binary mixtures with ethyl benzene.

Similarly, Mathur and Majumder (2010) investigated the degradation kinetics of BTEX compounds and phenol as a single and mixed substrate using *P.putida*. The authors observed a similar negative effect of competitive inhibition on the degradation of benzene in the presence of other BTEX compounds. In the two previous studies reported above, pure cultures were used which could have contributed to the negative effect reported by these authors. Perhaps, one possible reason might be due to the lack of highly versatile aromatic degrading enzymes that was capable of degrading the mixed substrates efficiently, considering the degree of recalcitrance, molecular structure and molecular weight of substrates involved. Other performance factors such as increased performance stability, resistance to foreign/ non-indigenous microorganisms among others, could also be associated with the performance of this adapted mixed culture in MFC systems. This suggests the added advantage of adapted anaerobic microbial consortia over pure cultures from an operational standpoint in the effective treatment of oil-contaminated environments.

The result of this study indicates the possibility of achieving over 95 % degradation efficiency in hydrocarbon mixtures, using adapted anaerobic microbial consortium, in a repeatable and consistent fashion during fed-batch MFC operation. The findings from this study recommend the potential use of MFC technology in enhancing degradation efficiency of petroleum hydrocarbons especially in subsurface environments in a cost effective and sustainable manner.

4.2.1.2 Electrochemical performance during MFC fed-batch operation.

Electrochemical performances of the fed-batch MFC operated over 6 cycles were fairly stable as shown in Table 4.1 and there was no significant difference ($p<0.05$) in the power outputs over the cycles of operation. Low electrochemical performances (in terms of power output and coulombic efficiency) observed in this study are similar to those reported by several authors that have investigated the treatment of petroleum hydrocarbons in MFCs (Morris and Jin, 2012; Wu et al., 2013; Chandrasekhar and Venkata, 2012). In a study on the anaerobic biodegradation of diesel in MFCs, Morris et al (2009) reported low peak voltage output of 50-65 mV but with enhanced degradation efficiency of 82 % compared to an anaerobically incubated control cell (31 %). Similarly, Wu et al (2013) reported maximum power density of 0.028 to 2.1 mWm^{-2} (using potassium ferricyanide as catholyte) as catholyte concentration was increased from 0 to 200 mM respectively.

Table 4.1: Electrochemical performances of petroleum hydrocarbon degradation during MFC fed-batch operation using adapted anaerobic microbial consortia. Values are means of duplicate experiments ± SD.

Cycle	Power density (mWm^{-2})	Peak voltage outputs (mV)	Coulombic efficiency (%)
CYC1	0.35±0.06	48.87±1.03	0.37±0.01
CYC2	0.57±0.01	45.20±1.08	0.22±0.06
CYC3	0.49±0.06	47.71±0.49	0.28±0.09
CYC4	0.47±0.01	48.89±1.87	0.38±0.07
CYC5	0.55±0.02	40.27±1.52	0.27±0.04
CYC6	0.91±0.08	48.92±0.21	0.39±0.03

Possible reasons for observed low electrochemical performances could be due to the nature of the substrates, large internal resistance, and partial oxidation of substrate leading to incomplete release of electrons, oxygen ingression into anode or substrate crossover. Simple substrates like glucose, acetate and other readily oxidisable substrates gave higher electrochemical performances than complex ones like petroleum hydrocarbons (Rosenbaum et al., 2011; Rabaey et al., 2003; Kim and Lee, 2010). Power production appears not to have negatively influenced biodegradation efficiency as highlighted above. Though power generation is low, it could still be used to power up low-power wireless sensors used in biomonitoring applications (Donovan et al., 2011).

4.2.2 Effect of salinity on overall system performance

In this study, salt concentrations 0.5, 1.0, 1.5, 2.0 and 2.5 % (w/v NaCl) were used in the anodic chamber of the MFCs in order to evaluate its influence on system performance. Results indicated that good electrochemical and degradation performance can be maintained up to 1.5 % NaCl. Optimum MFC performance was recorded at moderate salinity (1 % w/v NaCl) with both maximum power and voltage outputs improved by about 2 fold ($p<0.05$) compared to the initial salt concentration (0.1 % w/v NaCl) but decreased rapidly when salinity was increased to 2.5 % w/v (Figure 4.2). There was also a strong correlation between the power density and salinity ($r = 0.98$, $p<0.01$) which implies a significant impact that changes in salinity could have on MFC perfomance (as indicated in Table 4.2). It further suggest that salinity could be one of the most critical environmental parameters that could significantly influence operational conditions be it *ex situ*/*in situ* applications.

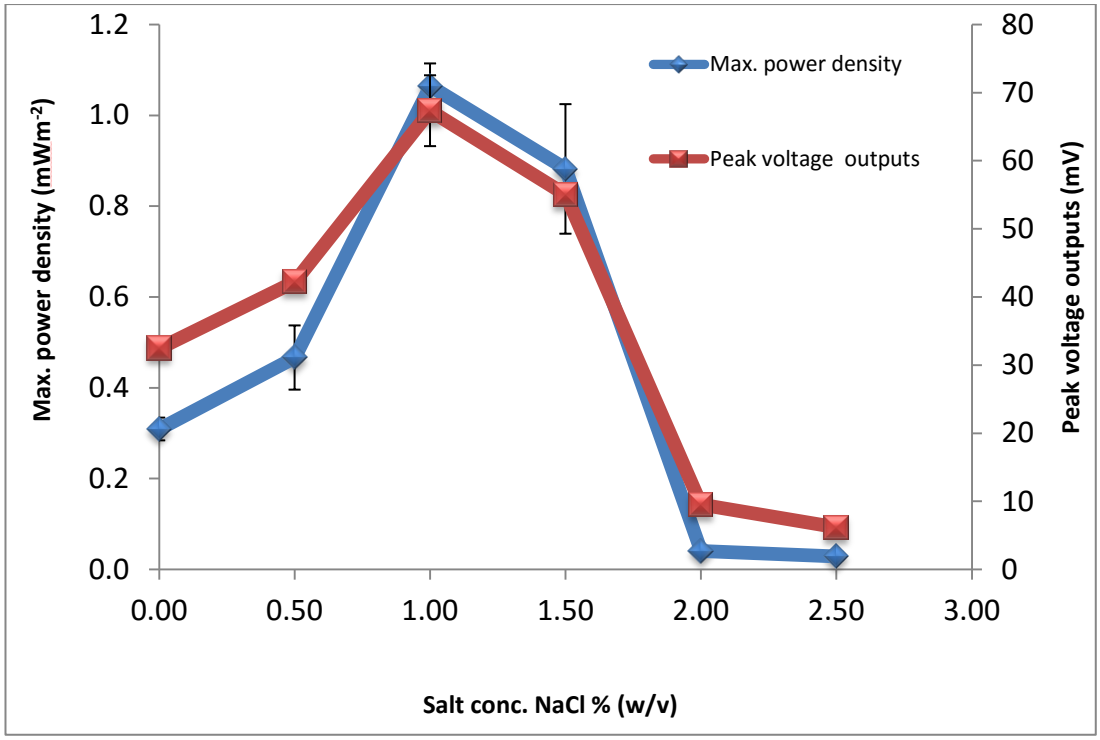


Figure 4.2: The effect of salinity on electrochemical performance of the MFC system at temp. 30℃ using adapted anaerobic microbial consortia.. Values are means of duplicate experiments ± SD.

Degradation and COD removal efficiencies increased gradually with increase in salinity till it peaked at 1 % (w/v NaCl) then dropped afterwards (Figure 4.3). The observed decrease in MFC performance when salinity was raised to 2.5 % w/v could likely be due to the dehydration of anodophilic cells at such relatively moderate saline condition (Lefebvre et al., 2012b). This appears to have adversely affected the physiology of the anaerobic microbial consortia and thus resulted into slower electron transfer rates at such high ionic strengths.

Minai-Tehrani et al (2009), in a similar study, investigated the effect of salinity on PAH biodegradation in oil-contaminated soil. The authors reported a sharp decrease in PAH removal efficency in oil-contaminated samples when salt concentration was raised from 1 % to 5 % NaCl. In another study, Lefebvre et al (2012b) reported that

higher NaCl concentration above 2 % was detrimental to overall system performance of an MFC fed with acetate. Increasing salt concentrations in MFCs are expected to result into corresponding rise in the ionic conductivity of the anolyte thereby decreasing the internal resistance and overpotential of the anode (Lefebvre et al., 2012b; Logan, 2008).

Table 4.2: Correlation analysis showing the effect of two factors (salinity and temperature) on MFC performance.

	COD removal (%)	Power density (mWm^{-2})	Petroleum hydrocarbon removal (%)		Coulombic efficiency (%)
			Phenanthrene	Benzene	
Salinity	**0.79**	**0.98**	**0.88**	**0.63**	**N.D**
Temperature	**0.94**	**0.90**	**0.96**	**0.99**	**0.92**

Colour code for correlation: Red- Very strongly positive correlation; Blue- Strongly positive correlation. ND- No data given.

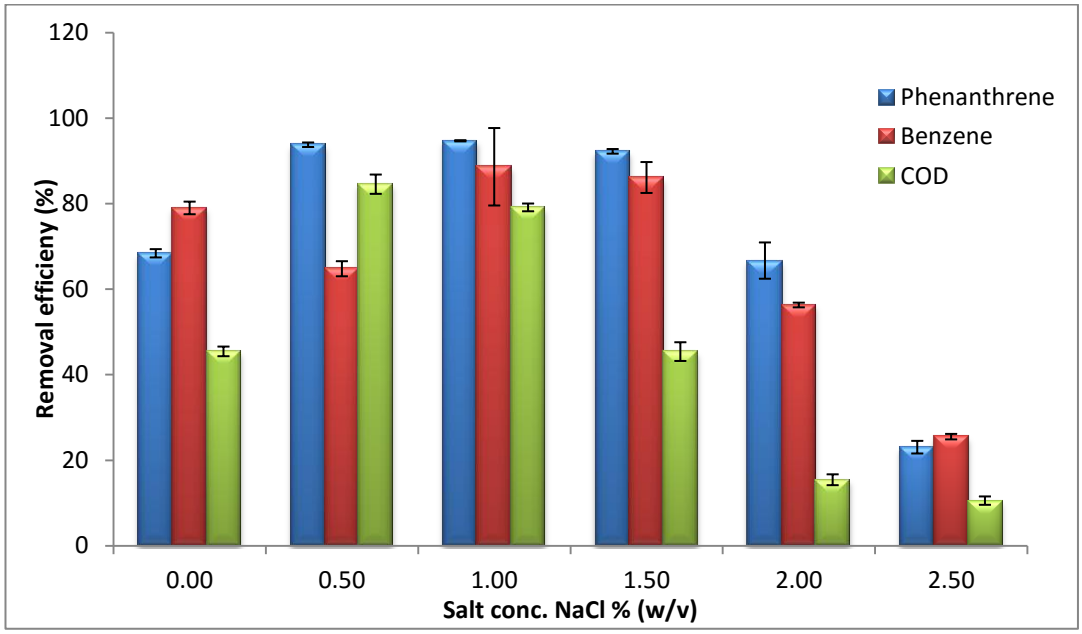


Figure 4.3: Effect of salt concentrations on COD removal and degradation efficencies during MFC operation (at 30℃) using adapted anaerobic microbial consortia. Values are means of duplicate experiments ± SD.

These changes could result in an improvement in electrochemical performance of the system; however, in highly saline conditions (as reported in this study) microbial metabolism is inhibited thus resulting into low MFC performance. However, halo-tolerant consortia can be used inMFCs at high saline environment. Findings from this study suggest that optimum MFC performance could be achieved when the MFC is operated at such moderate saline conditions (1 % w/v) such as in groundwater environments and sediments. In high saline environments, the use of halo-tolerant bacteria in marine environment (with salt concentration up to 3.5 % NaCl w/v) will be preferable.

4.2.3 Effect of operating temperature on system performance.

The influence of operating temperature (ranging from 20℃ to 50℃) on degradation and electrochemical performances was investigated (Table 4.3). Degradation efficiency and maximum power density increased with increasing temperature (i.e. between 20℃ and 40℃), afterwards, a sharp decrease in MFC perfomance was observed at 50℃ (Figure 4.4). Degradation rates, maximum power density and coulombic efficiency were all improved by approximately 2 fold at 40^{o}C (Table 4.3). Correlation studies also indicated a good relationship ($r = 0.97$, $p<0.01$) between change in temperature (i.e. between 20℃ and 40℃) and other performance-based parameters such as degradation rates, maximum power density and coulombic efficiency (Table 4.2). Decrease in MFC performance at operating temperatures higher than 40℃ may be due to loss of microbial activity resulting from non-optimal MFC operating condition. This implies that the adapted microbial consortia used in this study (see section 2.3) could not tolerate thermophilic conditions.

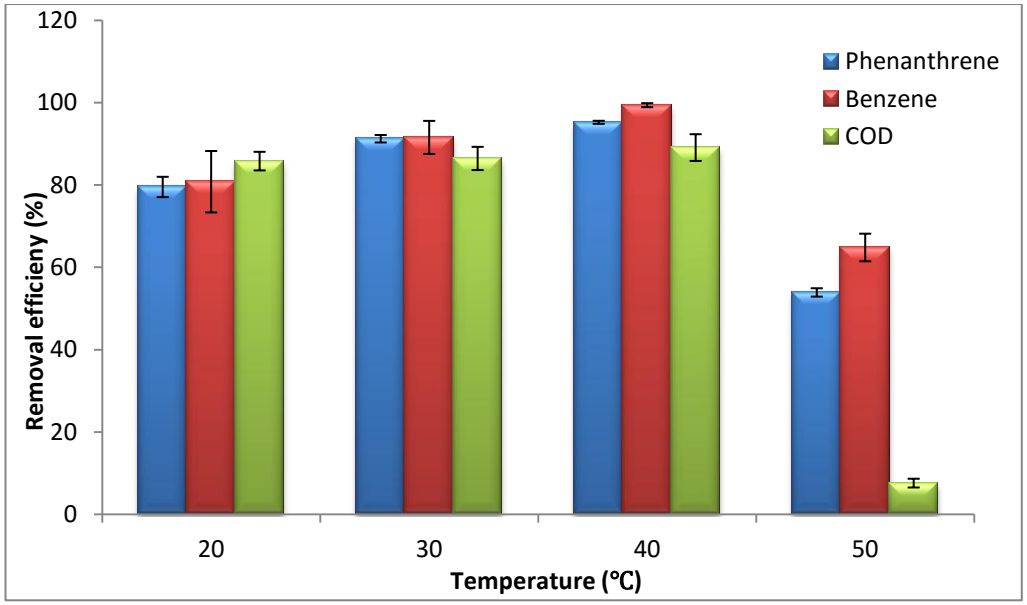


Figure 4.4: Effect of operating temperature on COD removal and degradation efficencies during MFC operation using adapted anaerobic microbial consortia. Values are means of duplicate experiments ± SD.

The adapted microbial consortium appears to prefer MFCs operated at mesophilic temperatures for petroleum hydrocarbon degradation. Hence, we would not recommend its technical applications beyond the tolerable temperature limit (i.e. 40℃). At higher temperatures (i.e. above 40℃), thermo-tolerant consortia can be adopted as preferable choice in such thermophilic environments or bioreactors.

Table 4.3: The effect of operating temperature on electrochemical performance of the MFC system using adapted anaerobic microbial consortia.. Values are means of duplicate experiments ± SD.

Operating temperature (℃)	Degradation rate (mg $L^{-1}d^{-1}$)		Maximum Power density (mWm^{-2})	Average current density (mAm^{-2})	Coulombic efficiency (%)
	Phenanthrene	Benzene			
20	23.14± 1.51	15.60±1.05	0.60 ± 0.03	2.22 ± 0.25	0.19 ±0.11
30	33.82± 2.83	22.62± 0.55	0.72 ± 0.04	4.89 ± 0.35	0.40 ±0.01
40	56.96± 3.81	39.78± 1.67	1.15 ± 0.18	8.87 ± 0.85	1.94 ±0.23
50	19.58± 1.20	23.40± 0.74	0.26 ± 0.01	0.86 ± 0.14	0.37 ±0.10

At higher temperatures, activation energy required to drive the oxidation process is lowered. Increases in operating temperature could have multiple effects including the enhancement of enzymatic kinetic rates, and an increase in the conductivity of the anolyte medium which may have contributed to reduction in internal resistance of the cells (Kim et al., 2005; Liu et al., 2004b). All these generally have a positive effect on the system perfomance regarding degradation rates, power generation and CE % as observed in this study (as temperature was raised from 20℃ to 40℃).

Notably, the temperature range tested was not linked solely to the microbial activity but was rather about the efficiency/performance of the microbial fuel cell in general. In this study, MFC performance was optimized at an operating temperature of 40^{o}C. This suggests a potential application of MFC system in tropical and hot-climate regions (like Africa) in the clean-up of oil-contaminated sites at both subsurface and groundwater environments.

4.2.4 Effect of redox mediators.

The effect of redox mediators on degradation efficiencies of petroleum hydrocarbons and MFC performance is shown in Figure 4.5 and Table 4.4 respectively. In the presence of redox mediators, the degradation performance could be maintained while improving electrochemical performance of the MFC system. Notably, one of the redox mediators, riboflavin, impacted power generation and coulombic efficiencies (CEs) significantly (Table 4.4). The maximum power density and CE % were raised by 30 fold and 3 fold respectively compared to mediator-free incubation when anolyte medium was supplemented with 30 µM riboflavin.

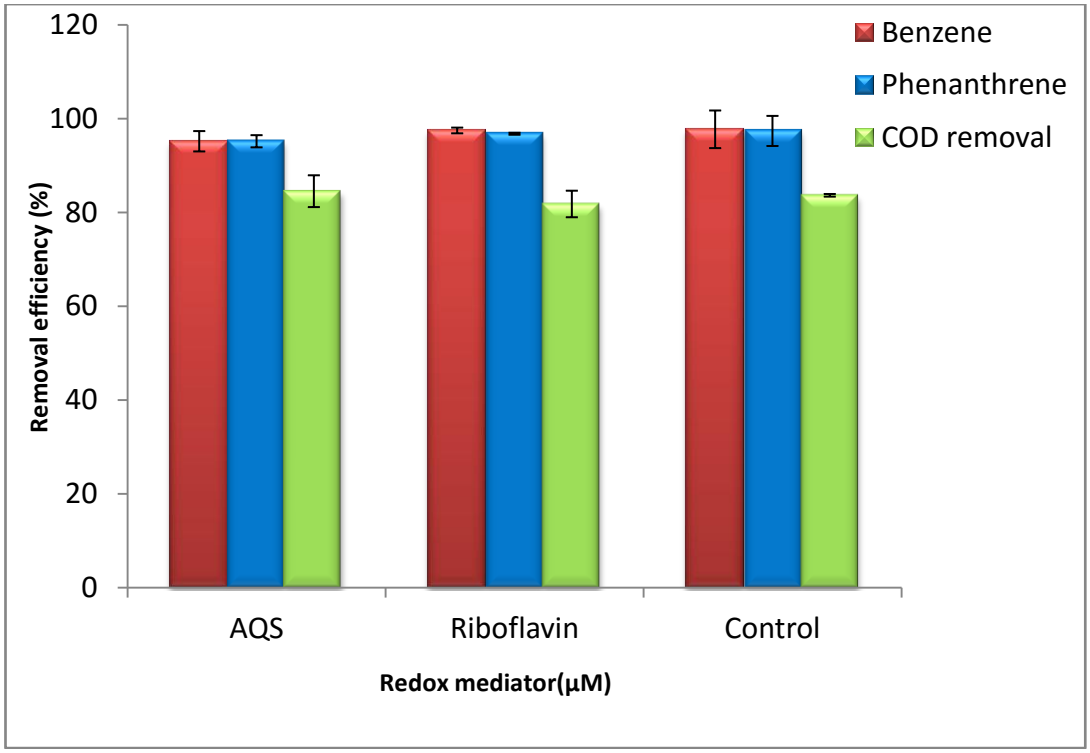


Figure 4.5: Effect of redox mediators on COD removal and degradation efficencies during MFC operation using adapted anaerobic microbial consortia. Values are means of duplicate experiments ± SD.

Additon of redox mediator has been shown to enhance electron transfer (Keck et al., 2002; Hawkes et al., 2010). In a related study, Park and Zeikus (2000) have demonstrated that electricity generation in a glucose-fed MFC was enhanced by about 10 fold than mediator-free MFC when neutral red (a redox mediator) was added to the anolyte medium.

Table 4.4: Electrochemical performances of two different redox mediators in an MFC using adapted anaerobic microbial consortia. Values are means of duplicate experiments ± SD.

Redox mediator (30 µM)	**Max. power density (mWm^{-2})**	**Peak voltage output (mV)**	**Coulombic efficiency (%)**	**COD removal efficiency (%)**
AQS	0.54±0.05	66.61±4.90	0.52±0.04	84.55±0.39
Riboflavin	26.17±0.08	265.33±1.19	1.44±0.08	81.82±1.83
Control (no redox mediator)	0.47±0.01	47.50±0.54	0.44±0.03	83.67±0.28

Dos Santos et al (2004) demonstrated that decolourisation rates of dyes in synthetic wastewater at thermophlic conditions could be increased by 8 fold in the presence of riboflavin compared with mediator-free control. High decolourisation rates were achieved due to enhancement in electron transfer to the dyes (in the presence of the redox mediator), thus increasing colour reduction/removal. Based on our results, riboflavin was far better than AQS with regards to their electrochemical performances. This could be explained by the differences in their redox potentials and chemical structures. Riboflavin, a flavin-based compounds (having diverse functional groups such as ketones and hydroxyl groups), has redox potential of -208 mV(vs. NHE) whereas AQS is a quinone-based compounds having redox potential of -225 mV (vs. NHE) (Dos Santos et al., 2004). For effective electron transfer, a higher redox potential of the redox mediator would thermodynamically favour the microrganism for its growth and maintenance of microorganisms (Logan, 2008). The results from this study suggest the use of riboflavin as the preferred redox mediator in optimising power generation while maintaining good degradation efficiency of petroleum hydrocarbons.

4.2.5 Effect of chemical catholytes on cathodic MFC performance

Three chemical catholytes namely, potassium bromate, potassium persulphate and calcium hypochlorite were tested for their potentials as cathodic electron acceptors in lieu of Pt-catalysed cathode. The degradation performance of petroleum hydrocarbon mixture (30 mg L^{-1} phenanthrene and 200 mg L^{-1} benzene) using different chemical catholytes as terminal electron acceptor is shown in Figure 4.6. Out of the three electron acceptors tested, potassium bromate gave the best MFC performance while calcium hypochlorite showed the least. Notably, the under performance of the hypochlorite might be due to its chemical instability or the production of chlorine

gas which could have diffused through the PEM (Proton Exchange Membrane) to the anode thus limiting microbial kinetics. Due to the loss of chlorine gas from the cathode, a possible increase in catholyte's pH may occur since chlorine gas was the active oxidants released by the hypochlorite in aqueous medium.

The suggested reasons aforementioned could have led to high over potential at the cathode, hence limiting overall system performance, especially in terms of power outputs. In a similar study, Momoh and Naeyor (2010) also reported the evolution of chlorine gas in cathode chamber of the MFC when a bleaching powder, 20 g L^{-1} calcium hypochlorite ($Ca(OCl)_2$), was used as cathodic electron acceptor.

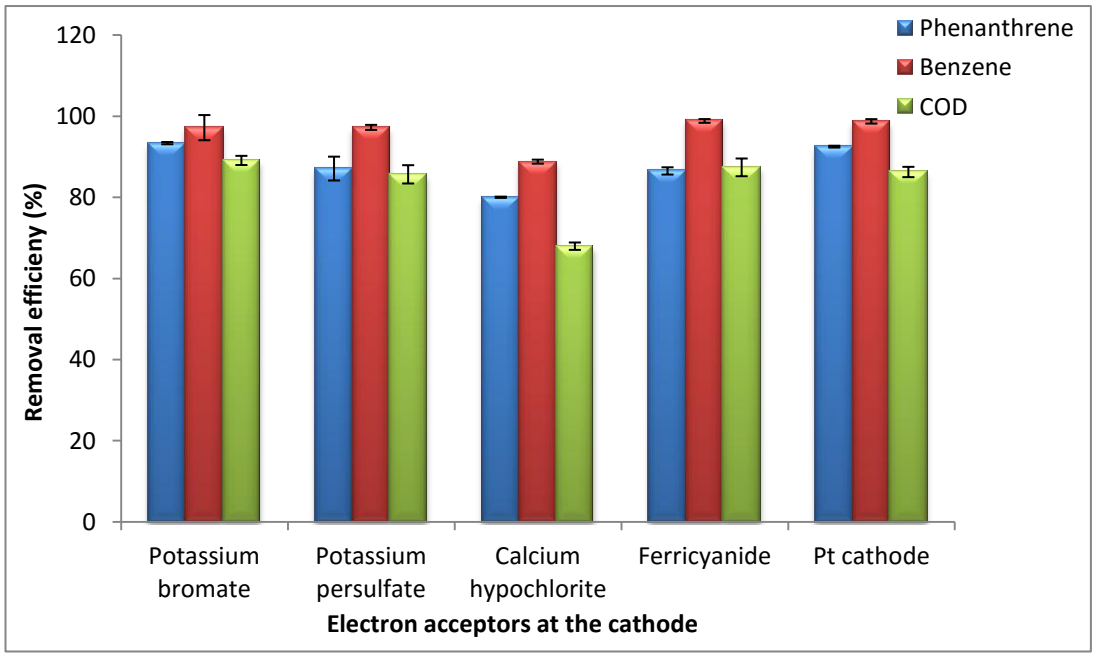


Figure 4.6: Effect of electron acceptors on COD removal and degradation efficiencies during MFC operation using adapted anaerobic microbial consortia. Values are means of duplicate experiments ± SD.

Electrochemical performance in terms of power density and voltage output was increased by about 10 fold and 2.5 fold respectively compared to platinised cathode when potassium bromate was used as catholyte (Figure 4.7). Meanwhile good degradation performance was also maintained at removal efficiency of 89.1 % and

85.5 % for potassium bromate and potassium persulfate respectively, which is slightly higher than Pt-cathode (84 %). Observed increment in electrochemical performance of the electron acceptors (i.e. potassium bromate and potassium persulphate) could be attributed to their high redox potentials and hydrophilic nature. Oxygen solubility in water is very low (about 4-6 mg L^{-1} at room (i.e. 25℃) temperature) compared to these water-soluble electron acceptors. In addition, poor contact between the oxygen molecules and cathode electrode' surface and coupled with slow reaction kinetics of ORR on carbon electrodes are also a potential contributing factor to low cathodic performance observed in MFCs. Theoretical standard redox potentials of potassium bromate and potassium persulphate are +1.44 V and +2.01 V vs NHE respectively which are also higher than of oxygen in the presence of Pt catalyst (+0.820V vs NHE).

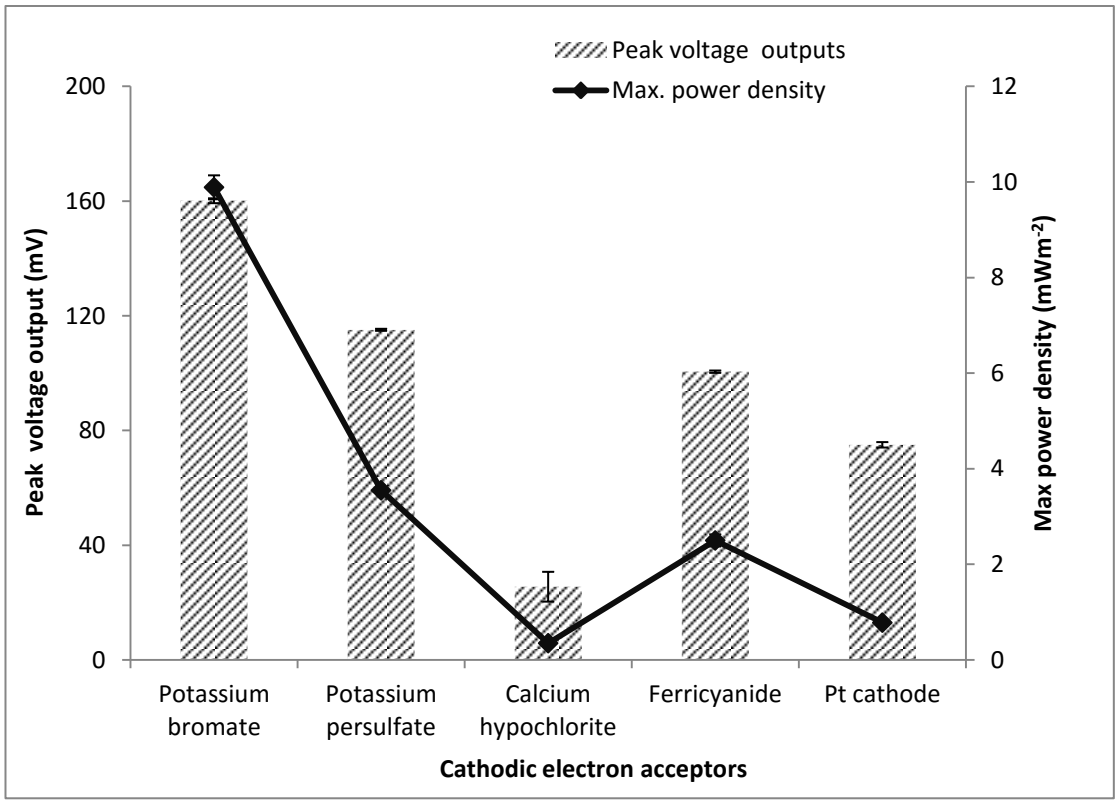


Figure 4.7: The electrochemical performance of the MFC using different electron acceptors at the cathode (Ext. resistance, R= 1 kΩ). Values are means of duplicate experiments ± SD.

High cost of MFCs as reported by several authors was due to the use of platinum in the cathode in order to enhance oxygen reduction reaction (ORR) at the cathode (You et al., 2006b; Hawkes et al., 2010; Rabaey et al., 2004). Because of the high cost of platinum and possible catalyst poisoning or wash off (especially in environmental matrices), its use in large scale MFC operations is largely limited. Consequently, there is a growing interest among researchers in this area to seek a more sustainable approach or a suitable replacement (for MFC Pt-coated cathode) in order to drive down cost while maintaining good cathodic performance. To this end, potassium bromate could be a replacement for Pt cathode due to its low cost and availability as pollutant from ozone-treated wastewater. The cost of bromate which is £0.20/gram is 600 times less than the cost of 1g of platinum powder (i.e. £120/gram). In other words, bromate contaminated wastewater can be treated in the cathode while concomitantly treating wastewater or other industrial effluents in the anode chamber of MFCs coupled with power generation. Electron released from the anode via an external circuit can be accepted by bromate ions thereby converting them to bromide ions. Bromate can be applied to contaminated soil or groundwater systems either exogenously or endogenously (i.e. bromate present in such environment as pollutants). To the best of authors' knowledge, there is no regulation as regards the use of bromate in MFC systems for remedial applications.

Bromide ions are generally known to pose no immediate health risk because they are very less toxic. Bromate contamination, has also been reported in groundwater, stagnant ponds/lakes and marine environment with moderately high chloride ions concentrations (Zhao et al., 2012; Von Gunten et al., 1996; Bao et al., 1999). In such environments, bromide ions are also usually present and can easily undergo photo-activation leading to the formation of bromate ions (Zhao et al., 2012; Lefebvre et al.,

1995). Photochemical oxidation /activation, ozonation or chlorination of wastewater and drinking water in treatment facilities and also in water bodies resulted into the accumulation of bromate in the environment.

This study suggests the use of bromate in MFCs as catholytes or electron acceptors for significant improvement in power production in lieu of expensive and non-renewable metal catalyst.

4.2.6 The influence of non-ionic surfactants on PHE bioavailability in MFCs

The effects of two different non-ionic surfactants (namely Tween 80 and Triton X100) on MFC performance at different surfactant concentrations is shown in Table 4.5. Degradation efficiencies and rates generally decreased with increasing concentration of each surfactant.

Table 4.5: Degradation and electrochemical performances of MFCs fed with different surfactants using adapted microbial consortia. Values are means of duplicate experiments ± SD.

Surfactants		**Maximum Power density (mWm^{-2})**	**Degradation efficiency (%)**	**Degradation rate ($mg\ L^{-1}d^{-1}$)**
Tween 80	20CMC	0.57 ± 0.14	78.75 ± 3.22	4.73 ± 0.80
	200CMC	0.51 ± 0.03	6.10 ± 1.58	0.36 ± 0.06
	600CMC	0.03 ± 0.002	3.90 ± 0.23	0.23 ± 0.07
Triton X100	20CMC	1.23 ± 0.06	30.96 ± 1.41	1.86 ± 0.19
	200CMC	0.85 ± 0.08	26.67 ± 0.99	1.26 ±0.02
	600CMC	0.79 ± 0.12	23.33 ± 3.22	1.44 ± 0.11
PHE only (control)	No Surfactant	0.71 ± 0.10	94.03 ± 2.28	5.64 ± 0.89

However, degradation efficiencies and rates deteriorated at a faster pace with increasing concentrations of Tween 80 compared to Triton X100. The adsorbed phenanthrene (PHE) on the electrode also decreased with increasing surfactant concentrations, suggesting the efficacy of these surfactants in enhancing PHE availability in aqeuous phase for possible microbial utilization (Figure 4.8).

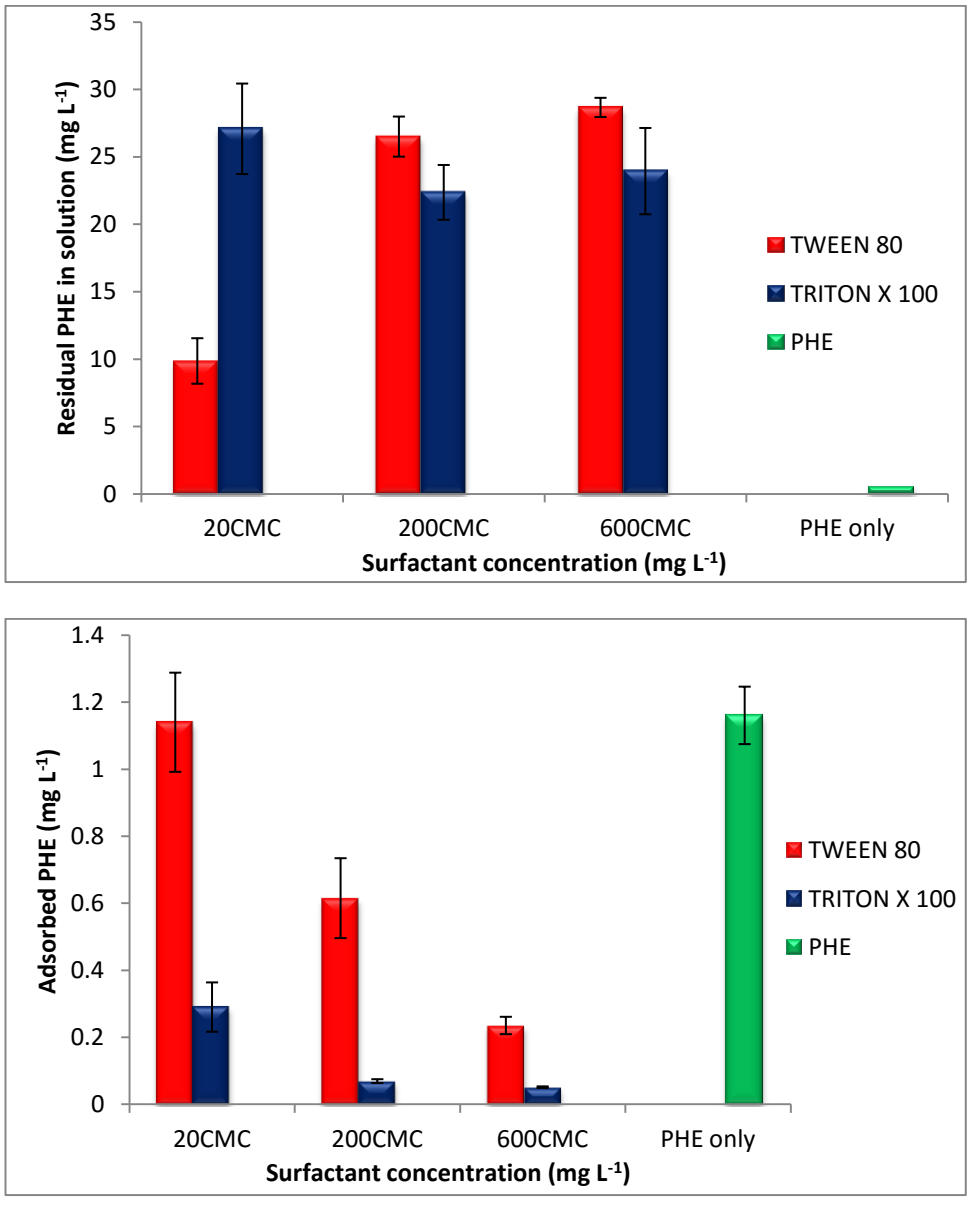


Figure 4.8: (A) Residual PHE (phenanthrene) present in the aqueous phase about 24 h after MFC operation began (B) Adsorbed PHE on the electrode after MFC operation for different concentrations of surfactants tested using adapted microbial consortia as inoculum source. Values are means of duplicate experiments ± SD.

From the observation above, it can be deduced that Triton X100 could be much better surfactant in improving PHE availability in the aqueous phase than Tween 80. Surfactants above their critcal micelle concentration (CMC) could solubize hydrophobic contaminants into their micelles, hence may enchance the biodegradation of these contaminants (Chen and Wong 2006; Kim et al., 2001).

Although an increase in residual PHE concentration was observed in the presence of the two surfactants (Figure 4.8 A), this apparent increase in PHE concentration has not led to an increase in the amount of bioavailabile phenanthrene, in the aqueous phase, for possible utilisation by microorganisms present in the anode chamber of the MFC. Perhaps, one possible explanation for this observation could be the non-bioavailability of PHE partitioned into the micellar-phase, an additional toxic effect of the surfactant on microbial physiology and possible steric hindrance due to the bulkiness of the surfactants molecules (Cheng and Wong, 2006; Mulligan et al., 2001; Yang et al., 2003). At higher surfactant concentrations, almost all of the phenanthrene mass is in the micellar phase; and if not bioavailable, may slow down the degradation rates significantly as observed in this study (Table 4.5). The micellar-phase bioavailable fraction of phenanthrene might have probably decreased with an increasing surfactant concentrations (Yang et al., 2003). Degradation efficiencies decreased sharply with gradual increase in Tween 80 concentrations than in Triton X100 concentrations as observed in Table 4.5. This observed trend may likely be due to the decrease in bioavailable fraction of phenanthrene concentration in the micellar-phase coupled with toxicity effects from increasing surfactant concentrations which might be more pronounced in Tween 80 than Triton X100. Hence, this indicates that Tween 80 may probably be more toxic than Triton X 100

and thus not suitable for use in enhancing the treatment of hydrophobic hydrocarbons like phenanthrene in MFCs.

The hydrophobicity of the cell surface may also have a significant effect on the rate of transfer of phenanthrene from the micellar phase to the cell. The higher the degree of hydrophobicity of the bacterial cells, the more chances that the bacterial cells can access the dissolved phenanthrene fraction in the micellar phase. The findings that phenanthrene partitioned into the micellar phase of both surfacants was not directly bioavailable could be attributed to an excessive dilution by the surfactant phase. Yuan et al (2000) observed a strong inhibition of phenanthrene biodegradation in the presence of some non-ionic surfacants above their CMCs. Their findings support the observed trend in this present study.

However, with each surfactant, a significant inhibition was observed and this inhibition increased with increasing surfactant concentrations. Addition of surfactant increases the toxicity of the anode solution when compared with control with surfactant as observed in this study (Figure 4.9). Toxicity effects may have contributed to lower degradation efficiencies observed.

The toxicity of surfactant to microbial community present in the MFCs is related to the surfactants' lipophilicity (Bautista et al., 2009). The higher the HLB (Hydrophile-Lipophile Balance) values, the lower the toxicity effect of surfactant on microbial cells. The HLB values of Tween 80 and Triton X100 are 15 and 13.5 respectively (Tiehm, 1994). Surfactant toxicity stems from their ability to interact with cell membranes, thus increasing their permeability, and to bind to protein and inhibit

enzyme activity, among other effects (Cheng and Wong, 2006; Bautista et al., 2009). In this study, the surfactant with the highest lipophilicity, Tween 80, exhibited higher toxic effect (compared to the control) on microbial growth hence inhibiting overall MFC performance (Figure 4.9).

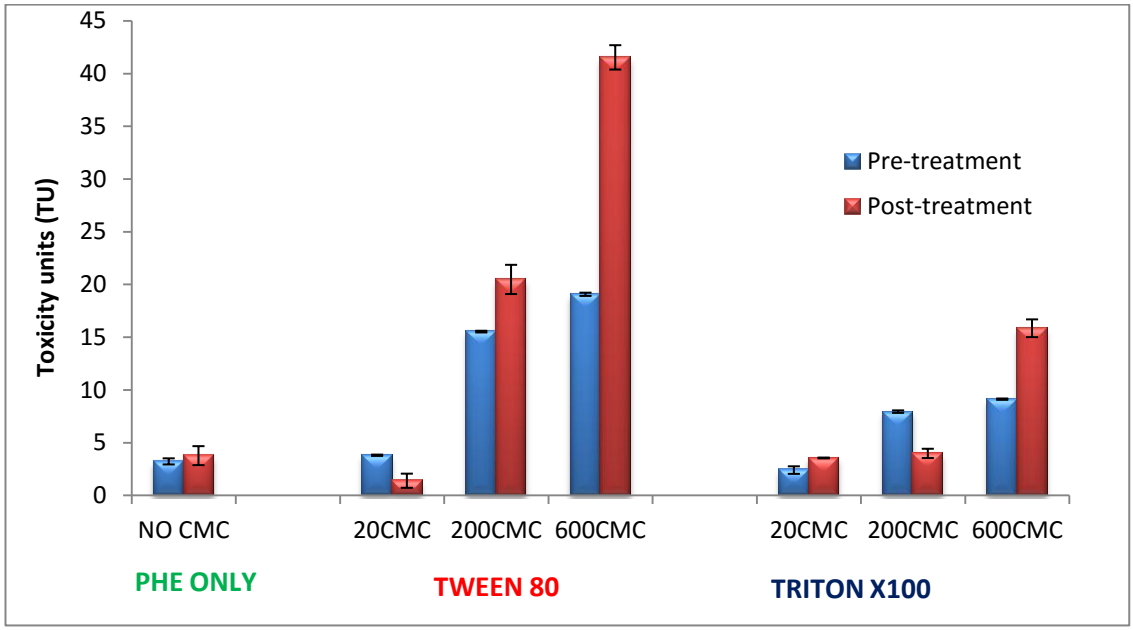


Figure 4.9: Toxicity response of two different surfactants at varying initial concentrations before and after treatment in a MFC using adapted microbial consortia as inoculum. The TU values are means of duplicate experiments ± SD.

From this study, it can be noted that Triton X100 has lower toxic effect relative to Tween 80; only 20 CMC of Triton X100 was required in order to achieve maximum PHE in aqueous phase. The addition of surfactant within the range tested increases apparent aqueous solubilty of PHE but did not enhance degradation efficency of phenanthrene when compared to a control with no surfactant addition. However, the addition of surfactants without toxicity may be a useful tool to accelerate bioremediation of contaminated areas.

4.2.7 Effect of external resistance as a tool in enhancing on MFC performance

COD removal and degradation efficiencies as function of external resistance (ranging from 100 Ω to 100 kΩ in an increasing order) is shown in Figure 4.10. Notably, the differences in degradation and COD removal efficiencies among external resistances were not statistically significant in all experiments ($P<0.05$, one way ANOVA with Tukey post-test).

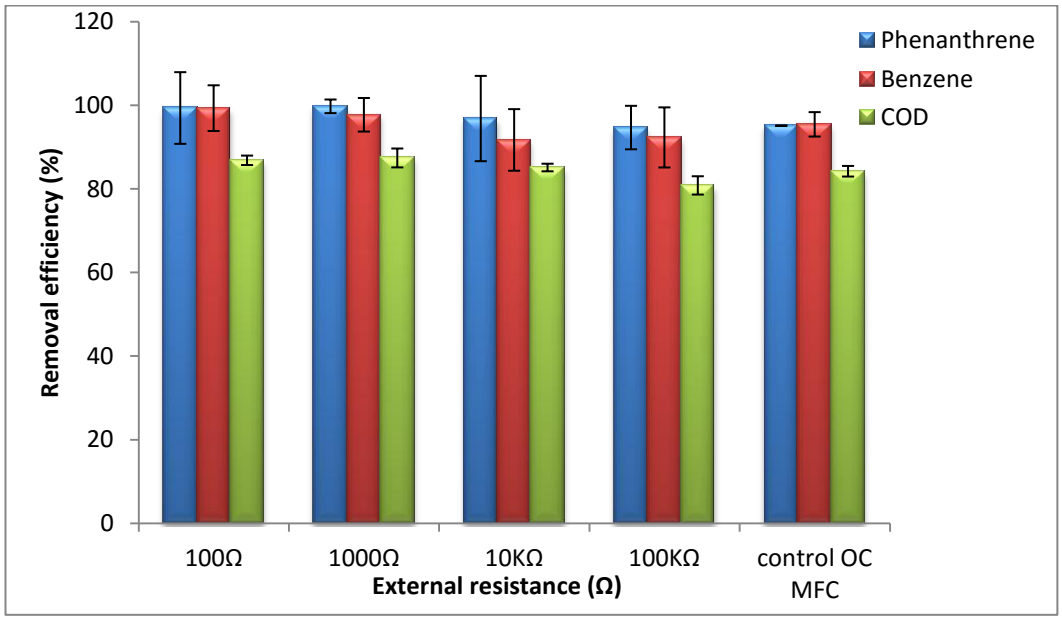


Figure 4.10: Degradation and COD reduction performance of MFC systems at various external resistances compared to open circuit control during 10 days of operation using adapted mixed culture at incubation temperature of 30±3℃.. Values are means of duplicate experiments ± SD.

However, one of our main long term goals in this study is to optimise power generation with minimal or insignificant impact on degradation/treatment efficiency. Maximum power density is a function of both the total internal resistance of the MFC system and the external resistance applied across it (Zhang and Liu, 2010). Power ouputs in MFCs can be enhanced by lowering the internal resistance within the cell which could be achieved by either reducing anodic – cathode size ratio or increasing the external resistance (Logan, 2008; Fernando et al., 2014a). In this study,

optimal power density of 10.09 mWm^{-2} was obtained at an applied external resistance of 100 kΩ (10 fold higher than power obtained at 100 Ω) while maintaining a good degradation performance (Figure 4.10 and Table 4.6) compared relatively to other external resistances and the control.

At higher external resistances (10 kΩ to 100 kΩ), lower anode potential obtained may alter the metabolic activities of the anodic microbial community, as previously demonstrated in some studies conducted by Picioreanu et al (2007) and Jung and Regan (2011). Anode potential might influence competition among electrogenic bacteria either directly via anode utilization (e.g. regulation of substrate conditions) or indirectly through microenvironmental and operating conditions (Jung and Regan, 2011; Fernando et al., 2014a). Lower anodic potentials favour *Geobacter* species and other low-potential respiratory anaerobes which can also be achieved at higher external resistances (Jung and Regan, 2011).

Table 4.6: Degradation and electrochemical performances of MFCs at different applied external resistances using adapted mixed culture at incubation temperature of 30±3℃.. The error bars represent the SD of the mean.

External resistance (Ω)	**Maximum Power density (mWm^{-2})**	**Peak current density (mAm^{-2})**	**COD removal efficiency (%)**	**Coulombic efficiency (%)**	**Internal resistance (Ω)**
100	0.47 ± 0.03	22.95 ± 0.85	86.86 ± 1.13	1.11 ±0.11	2160 ±42.6
1000	0.71 ± 0.07	13.35 ± 0.28	87.42 ± 2.26	0.44 ±0.23	990 ±13.5
10k	0.79 ± 0.05	3.86 ± 0.35	85.13 ± 0.91	0.15 ±0.09	517 ±8.20
100k	10.09 ± 0.14	1.28 ± 0.14	80.85 ± 2.19	0.08 ±0.10	470 ±34.65
Control (closed circuit MFC)	ND	ND	84.24 ± 1.27	ND	ND

Some bacterial species have been reported to possess the ability to easily adapt themselves to a specific potential by regulating redox enzymes expression and production of cofactors such as NADH/NAD^+ ratio (Gruning et al., 2014; Rabaey and Verstraete, 2005). The presence of different microbial species (including fermenters and methanogens) could have possibly provided different mechanisms for utilising organic contents in the MFC anode efficiently (Katuri et al., 2011). Menicucci et al (2006) reported that maximum sustainable current and power obtainable from an MFC system was greatly influenced by the applied external resistance, which agrees well with the findings in this study. Contrary to findings of this study and of other studies (Fernando et al., 2014a; Menicucci et al., 2006; Katuri et al., 2011), a previous study by Lyon et al (2010) found that external resistance has little or no significant effect on MFC power production.

Applied external resistance is an important parameter that can influence the potentials of both the anode and cathode significantly and might to a large extent, determine the bioelectrochemistry and variation of the microbial population in the MFC anode. External resistance affects the anode microbial community, power production, methane or CO_2 production and electron flow without externally applied (or poised) potentials from a potentiostat (Lyon et al., 2010; Katuri et al., 2011). This experimental evidence clearly indicates that optimal power production can be achieved at optimal external resistance without any sigificant deteriotation in the degradation performance of a given MFC system.

The total bacterial biomass in the MFC (suspended biomass and biomass attached on the electrode) as a function of external resistance is shown in Figure 4.11. Bacteria mass increased steadily with increasing external resistance. The biomass yield at low external resistances (100 and 1000 Ω) was significantly ($p<0.01$) lower than biomass

yields at higher external resistances (10 kΩ and 100 kΩ) and further corroborated by high coulombic efficiencies at high current densities (Table 4.6). At low external resistances (or high current densities), electrogenic processes by electrochemically active bacteria presumably predominated over the non-electrogenic processes and this may have resulted into a greater portion of COD consumed which were mostly converted to electrical current harvested by the anode. High coulombic efficiencies and current densities (as shown in Table 4.6) observed in this study at low external resistance further complement these findings. Our data on low biomass yield and high current densities observed at low external resistance are in agreement with the findings of the earlier study conducted by Katuri et al (2011). Although anaerobic processes generally yield low biomass, further decrease in biomass yield can be obtained in MFCs operated at very low external resistances (Speece, 2002). Low biomass/sludge yield is an added advantage since it accounts for about 25-65 % of total treatment plant operating costs especially in water industry (Liu and Tay, 2001).

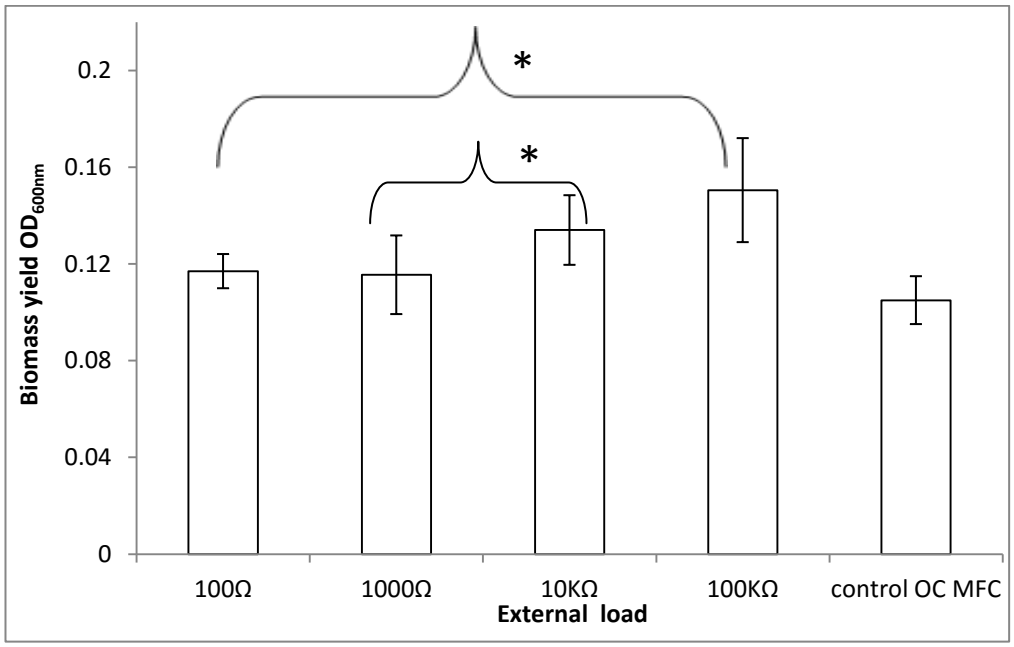


Figure 4.11: Total biomass yield measured at 600 nm under MFC operating with different external resistance and at open circuit (OC) MFC using adapted microbial consortia at incubation temperature of 30±3℃. Mean data from duplicate reactors with error bars (±SD). * indicates data is statistically significant at $p<0.01$.

Data from this study clearly indicates that varying external resistances had no significant effect on degradation efficiencies (for both phenanthrene and benzene) and COD removal efficiency but do impact significantly electrochemical parameters such as voltage ouput, power density and internal resistance. Control of external resistance is a simple method for studying bioelectrochemistry and exoelectrogenic ecology in MFCs and optimising electrochemical performance of MFC systems.

4.2.8 The effect of substrate concentrations

4.2.8.1 The effect of single substrate concentrations on MFC performance

The influence of initial benzene concentrations (ranging from 200-2000 mg L^{-1}) on MFC performance was conducted using adapted mixed culture (section 2.3). The effect of initial substrate concentrations on electrochemical and degradation performances are shown in Table 4.7. Results indicated that degradation rate of benzene increased with increase in the initial substrate concentrations. There was no significant difference in degradation efficiency at different benzene concentrations except at 2000 mg L^{-1}, showing the effect of substrate inhibition.

Table 4.7: MFC performances at different benzene concentrations using adapted anaerobic microbial consortia. Values are means of duplicate experiments ± SD.

Benzene concentration (mg L^{-1})	**Max. power density (mWm^{-2})**	**Peak voltage output (mV)**	**Coulombic efficiency (%)**	**Degradation rate (mg $L^{-1}h^{-1}$)**	**Degradation efficiency (%)**
200	0.82±0.14	50.17±4.83	1.07±0.04	4.99±0.07	90.65±2.21
500	0.57±0.08	39.02±1.41	0.79±0.01	6.55±0.12	94.02±2.28
1000	0.42±0.01	34.68±3.49	0.48±0.02	9.75±0.70	93.63±1.58
1500	0.31±0.05	30.43±1.52	0.55±0.02	12.95±1.02	93.43±1.23
2000	0.22±0.01	21.23±1.02	0.21±0.03	12.95±0.95	84.39±6.93
Control (Abiotic)	0.01±0.00	1.03±0.01	0.03±0.01	0.312±0.01	3.05±0.03

Notably, in agreement with the findings in the current study, Mathur and Majumder (2010) and Lee et al (2002) both reported an increase in specific degrdation rates for BTEX compounds at initial concentrations within the range of 10 to 400 mg L^{-1} and 23 to 70 μM respectively using a pure microbial culture.

Furthermore, there was a statistically significant ($p<0.05$) decrease in electrochemical performances with increasing benzene concentrations in general. This performance deteriorated about 4.5 fold and 13.5 fold respectively in terms of maximum power density and coulombic efficency when the initial concentration of benzene was raised from 200 mg L^{-1} to 2000 mg L^{-1}. The observed drop in electro-chemical performance with increases in substrate concentrations may be attributed to increase in substrate toxicity or substrate overloads. Decreases in microbial activity resulting from increasing toxicity and substrate inhibition which is associated with increasing benzene concentrations may have largely contributed to the trend observed in this study. Microbial degradation of benzene at high concentration (2000 mg L^{-1}) under anaerobic conditions have rarely been reported in the literature. High tolerance levels demonstrated by the adapted mixed culture used in this study suggest their use in the remediation of subsurface environments contaminated with high levels of benzene concentrations.

Moreover, a similar experiment was set-up to study another single substrate, phenanthrene with initial concentrations ranging from 10 - 100 mg L^{-1}. Table 4.8 shows the degradation and electrochemical performance for various initial concentrations of phenanthrene. We observed a significant increase in peak power densities and coulombic efficiencies (CE) % with increasing substrate concentrations.

A positive correlation existed between peak power densities, coulombic and degradation rates. However, there was a gradual decrease in COD removal efficiencies across various phenanthrene concentrations tested. The observed trend may be due to the formation of more biotransformed metabolites with higher molecular weights (resulting from the increased substrate overloading or rising substrate concentration). Substrate overcrowding or competitve inhibition could result in incomplete degradation or mineralisation to CO_2 or other metabolic products of low COD content. Phenanthrene used in this study was dissolved in methanol in order to enhance its solubility in the MFCs. The increment in the amount of methanol with corresponding rise in phenanthrene concentrations could have possibly contributed to the observed trend, especially at higher phenanthrene concentration where there was a clear divergence between COD removal efficiencies and degradation rates.

Table 4.8: Degradation and electrochemical performances of different initial phenanthrene concentrations. Values are means of duplicate experiments ± SD.

Phenanthrene concentration (mg L^{-1})	**Max. power density (mWm^{-2})**	**Degradation efficiency (%)**	**Coulombic efficiency (%)**	**Degradation rate (mg $L^{-1}d^{-1}$)**	**COD removal efficiency (%)**
10	0.31±0.14	97.37±2.83	0.21±0.04	3.20±0.01	77.91±2.87
20	0.47±0.06	98.72±1.41	0.36±0.01	6.59±0.15	65.46±2.08
30	0.84±0.03	98.43±1.31	0.46±0.05	7.30±0.75	58.57±3.21
50	0.81±0.01	97.91±8.49	0.47±0.01	6.94±0.68	56.44±1.07
100	1.07±0.11	96.74±3.54	0.52±0.09	13.88±1.29	47.08±2.91
Control (Abiotic)	0.01±0.00	3.05±0.01	0.01±0.00	0.71±0.01	4.81±0.03

Results revealed that there was no significant effect ($p>0.05$) on degradation efficiencies at different concentrations suggesting that the adapted mixed culture used could tolerate high concentrations of phenanthrene (as high as 100 mg L^{-1}). This observation probably suggests the presence of phenanthrene degrading microorganisms in the adapted mixed culture which gave it such a degree of tolerance at high substrate concentration. In a previous study, Zhao et al (2011) demonstrated that a phenanthrene degradaing bactericum, *Rhodopseudomonas palustris,* could tolerate up to 100 mg L^{-1} phenanthrene and anaerobically degraded 50 % of 50 mg L^{-1} phenanthrene over an incubation period of 10 days. In field applications, the ability of microorganisms (either indigineous or bioaugmented species) to utilise and tolerate high contaminant levels is pivotal to the success of any bioremediation technologies. Therefore, adapted mixed cultures with high tolerance to phenanthrene could be used for bioaugmentation purposes, especially in point-source contaminated sites.

4.2.9 Microbial community analysis of the adapted mixed culture from Fed-batch MFCs.

DGGE analysis of the 16S rRNA gene fragments from the anode community was conducted in order to identify bacterial community composition and have better understanding of microbial species involved in the anaerobic degradation of petroleum hdrocarbons in the anode and their interactions. Each DGGE band was considered to represent a single species. The variation in the postions of the DGGE bands in the DGGE profiles shown in Figure 4.12 suggest distinct microbial species present in the adapted mixed culture.

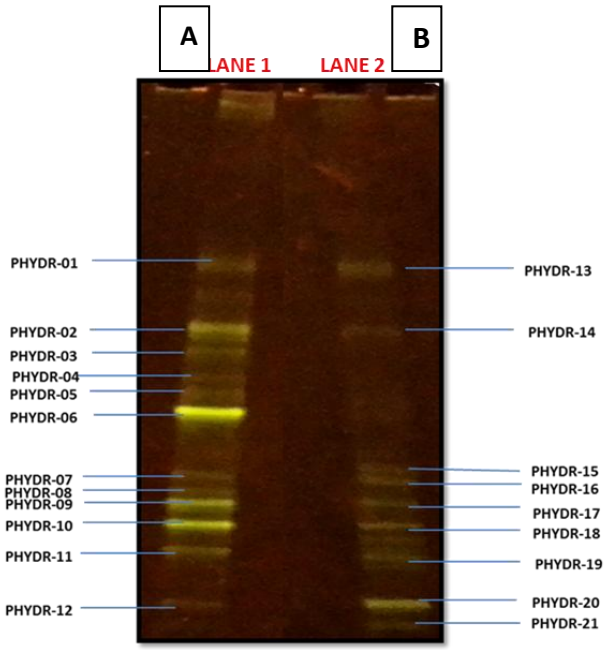


Figure 4.12: DGGE fingerprints of MFC acclimated microbial culture during petroleum hydrocarbon degradation in MFCs after approximately 60 days of fed-batch operation. Lanes 1 and 2 respectively were samples taken from duplicate MFCs (at temperature of 30℃).

Phylogentic affilations of the identifed bacterial species are shown in Table 4.9. The phylogentic analysis of 16s rDNA sequences revealed a high degree of bacterial diversity in the adapted mixed culture with majority of the sequences belonging to a bacterial phylum, *Proteobacteria* (59 %). Other bacteria phyla found included *Actinobacteria* (8 %), *Firmicutes* (17 %), *Crenarchaeota* (8 %) and *Thermotogae* (8 %) as shown in Figure 4.13A. This agrees with previous studies on petroleum hydrocarbon-contaminated bacterial communities conducted by some authors (Fernando et al., 2013; Morris et al., 2009; Lu et al., 2014a). *Proteobacteria* are exoelectrogens dominantly found in bacterial communities of the anode of MFCs, though some *firmicutes* may also be electrochemically active as previous reported by Fernando et al (2014a) and Lu et al (2014a). *Actinobacteria* are known to be abundant as a group of versatile hydrocarbon degraders with its relative abundance (8 %) of the total microbial population (Milton et al., 2010; Zhang et al., 2012c).

Table 4.9: Phylogenetic affiliations of the 16s rDNA sequences obtained from MFC s during its 60 days MFC operation (at 30± 5℃) based on the sequences in the 16s ribosomal DNA repository of NCBI.

Clones from MFC anodes (DGGE Bands)	Closest relative (% similarity)	GenBank Accession	Phylogenetic affiliation	Genus
PHYDR-01	*Pseudomonas pseudoalcaligenes* strain JCM 5968 (100)	NR_112065.1	Proteobacteria	Pseudomonas
PHYDR-02	*Klebsiella oxytoca* strain KCTC 1686 (100)	NR_102982.1	Proteobacteria	Klebsiella
PHYDR -03,07	*Vibrio vulnificus* strain ATCC 27562 (99)	NR_118930.1	Proteobacteria	Vibrio
PHYDR -04,05, 10	*Corynebacterium mycetoides* strain CIP 55.51 (98)	NR_119140.1	Actinobacteria	Corynebacterium
PHYDR -06,18	*Citrobacter freundii* strain LMG 3246 (99)	NR_117752.1	Proteobacteria	Citrobacter
PHYDR -08,13	*Vibrio parahaemolyticus* strain ATCC 17802 (100)	NR_118928.1	Proteobacteria	Vibrio halophile
PHYDR -09	*Clostridium sphenoides* strain DSM 632 (100)	NR_119035.1	Firmicutes	Clostridium
PHYDR -11	*Pseudomonas aeruginosa* strain SNP0614 (99)	NR_118644.1	Proteobacteria	Pseudomonas
PHYDR -12,21	*Clostridium polysaccharolyticum* strain DSM 1801 (97)	NR_119085.1	Firmicutes	Clostridium

PHYDR -14,17	*Pseudoalteromonas haloplanktis* strain ATCC 14393 (100)	NR_044837.2	Proteobacteria	Pseudoalteromonas
PHYDR -15	*Thermoproteus uzoniensis* strain 768-20 (100)	NR_102955.1	Crenarchaeota	Thermoproteus
PHYDR -16	*Marinitoga okinawensis* strain TFS10-5 (95)	NR_041466.1	Thermotogae	Marinitoga

At the genus level, three genera namely *Clostridium* (17 %), *Vibrio* (17 %) and *Pseudomonas* (17 %) were identitied as the dominant populations among the bacterial species found in the anode biofilm (Figure 4.13B). All these genera were known to be likely associated with petroleum hydrocarbon degradation. For example, some Gram-negative organisms such as *Pseudomonas* sp. and *Clostridium* sp. were reported to be responsible for extracellular electron transfer (EET) in MFCs (Rabaey and Verstraete, 2005; Fernando et al., 2013). *Pseudomonas aeruginosa* and *Citrobacter freundii* have been frequently reported to be responsible for power generation and are both model hydrocarbon-degrading bacteria in MFCs (Lu et al., 2014a; Rabaey et al., 2004; Friman et al., 2012). *Pseudomonas pseudoalcaligenes* (Figure 4.14) was recently reported by Ramalakshmi et al (2013) in a study to be responsible for electricity production in MFCs using corn steep liquor as substrate.

Thermoproteus uzoniensis strains were also known to be moderately thermoacidophile which are obligate anaerobes found to be sulfur-reducing bacteria in marine sediments (Mardanov et al, 2011). *Clostridium* species are well known for fermentative growth under thermophilic conditions (Zverlov et al., 2010) and could also be electrochemically active at low redox potentials in the MFC environment (Park et al., 2001; Fernando et al., 2013).

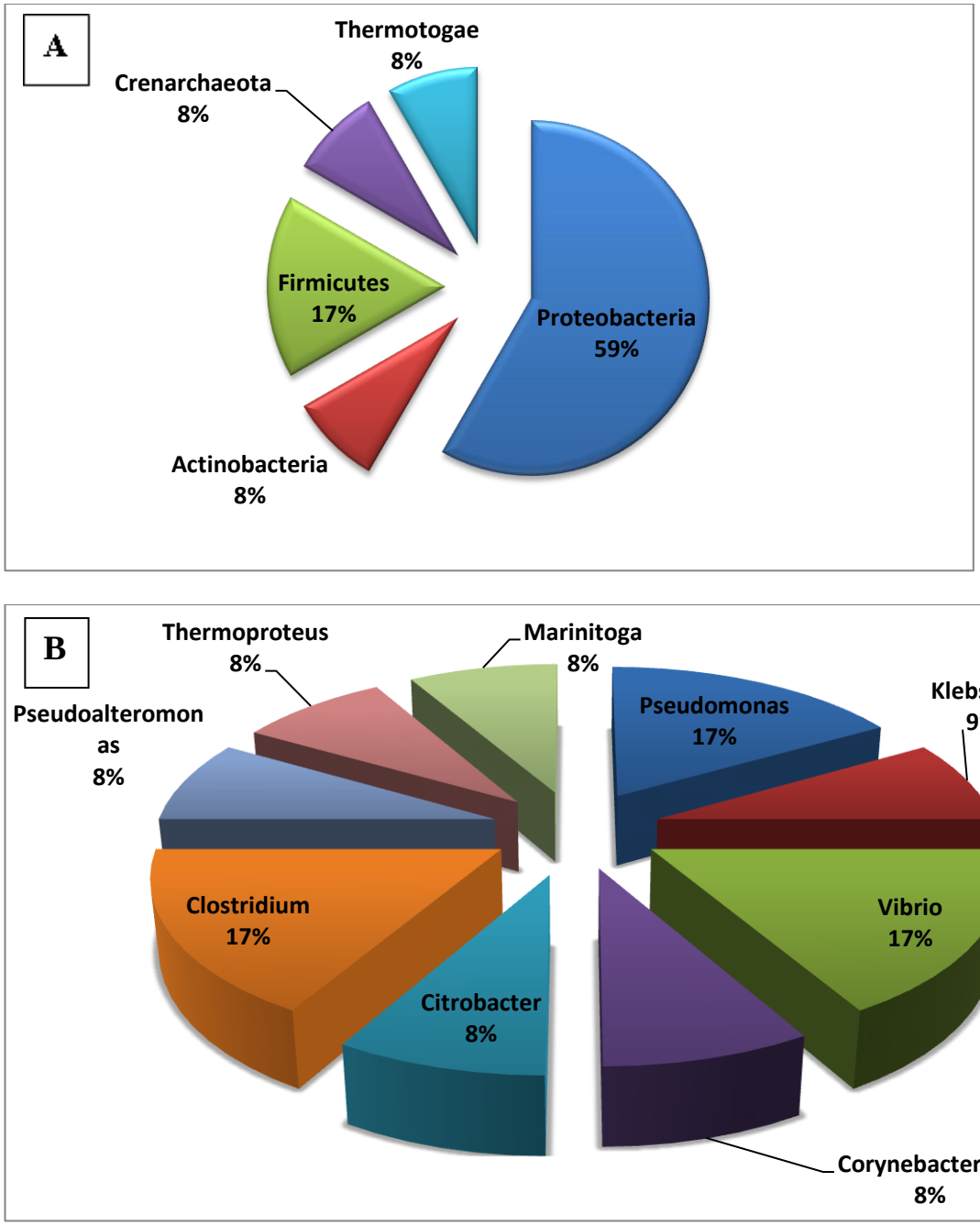


Figure 4.13: (A) Taxonomic classification of bacterial DNA sequences from communities of anode biofilms in MFCs at the phylum level and (B) the genus level distribution of the most dominant phylum. Relative abundance was defined as the number of sequences affiliated with that phylum or genus divided by the total sequence number of per sample.

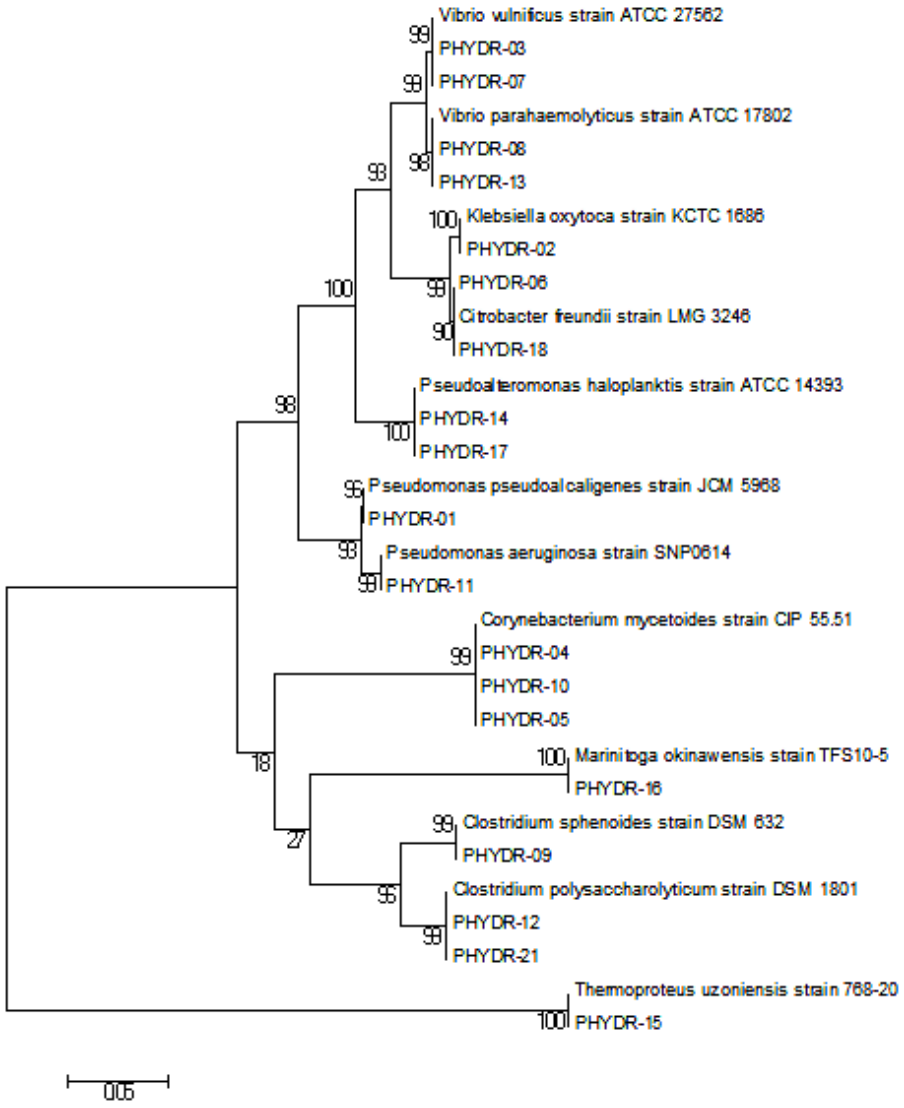


Figure 4.14: Phylogenetic tree of the bacterial communities selected in MFC anodes after 60 days of fed-batch operation, using adapted microbial consortia at 30± 5℃, were constructed using the neighbour joining method. Bootstrap values ≥50 % (from 1000 bootstrap replicates) are shown at the nodes of the tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.

The above results suggested the presence of both electrogenic and hydrocarbon degrading bacteria among the microbial community structure in the anode biofilm. The selection of other group of bacteria such as methanogens, fermenters among others and their syntrophic interactions with the hydrocarbon degrading bacteria and anodophiles could further enhance biodegradation and hence overall system

performance. The diversity of microbes (electrogenic and non-electrogenic bacteria) present on the electrode is an encouraging evidence that could suggest that MFC technology may prove useful for remediating a wide array of xenobiotics compounds. Therefore, the changes in the microbial community structure could be another factor that affects the performance of a MFC system.

4.2.10 The interactive effect of independent variables on MFC performance using factor using response surface methodology (RSM).

RSM is an efficient statistical tool which could be used to study the interactive factors and optimize process performance while taking into account the relationship between the whole and the part (Teng et al., 2010). As earlier mentioned in section 2.6.2, the independent variables, subject to statistical analysis, were selected through preliminary experiments as previously described in this chapter. Figure 4.15 presents the predicted versus actual plot of various response factors. Actual values were obtained from a particular run while the predicted values were calculated from the approximation used for the model. From Figure 4.15, data points in Figures 4.15A and 4.15B are not linearly fitted compared to Figure 4.15C in which there was a good line of best fit (i.e. it is linearly correlated). There was a very weakly negative correlation between COD removal efficiency and other factors (both independent variables and other response factors) as shown in Table 4.9.

However, there was a strong correlation between power density and external resistance. Changes in power density had very little impact in COD and TPH removal efficiencies. Increase in TPH removal does not necessarily imply an increase in COD removal efficiency as they are negatively correlated. Furthermore, the validation of the statistical models obtained for response factors, COD and TPH

removal using ANOVA suggests that these models were not statistically significant (Appendix 3). Hence, with evidence supported by the data presented in Table 4.10 and Figure 4.15, it is possible to conclude that the models may not be suitable for optimization or prediction purposes. However, the models can be used to navigate the design space or adequately describe interactions among independent factors within the design space since their adequate precisions values for COD and TPH removal are 4.08 and 4.41 respectively. A value greater than 4 is desirable as it indicates adequate signal from the model.

Table 4.10: Correlation matrix between the independent factors (A,B and C) and response factors (R1,R2 and R3) for the CCD experiments using RSM. The preferred choice of redox mediator used in this study was riboflavin.

	A – Salinity (%)	B – Ext. Resistance (%)	C– Redox mediator (µM)	R1– COD removal (%)	R2–Power density (mW/m^2)	R3 – TPH (%)
A – Salinity (%)	1.00	0.00	0.00	-0.24	0.09	-0.15
B – Ext. Resistance (%)	0.00	1.00	0.00	0.00	0.93	0.52
C– Redox Mediator (µM)	0.00	0.00	1.00	-0.36	0.01	-0.06
R1– COD removal (%)	-0.24	0.00	-0.36	1.00	0.16	-0.39
R2–Power density (mW/m^2)	0.09	0.93	0.01	0.16	1.00	0.26
R3 – TPH (%)	-0.15	0.52	-0.06	-0.39	0.26	1.00

Colour code: Light pink- weakly negative correlation; Light Blue- Weakly positive correlation; yellow - Very strongly positive correlation; Blue- Strongly positive correlation.

Figure 4.16 shows the perturbation plots of three different responses. In response surface designs, the perturbation plot shows how the response changes as each factor moves from the chosen reference point, with all other factors held constant at the reference value.

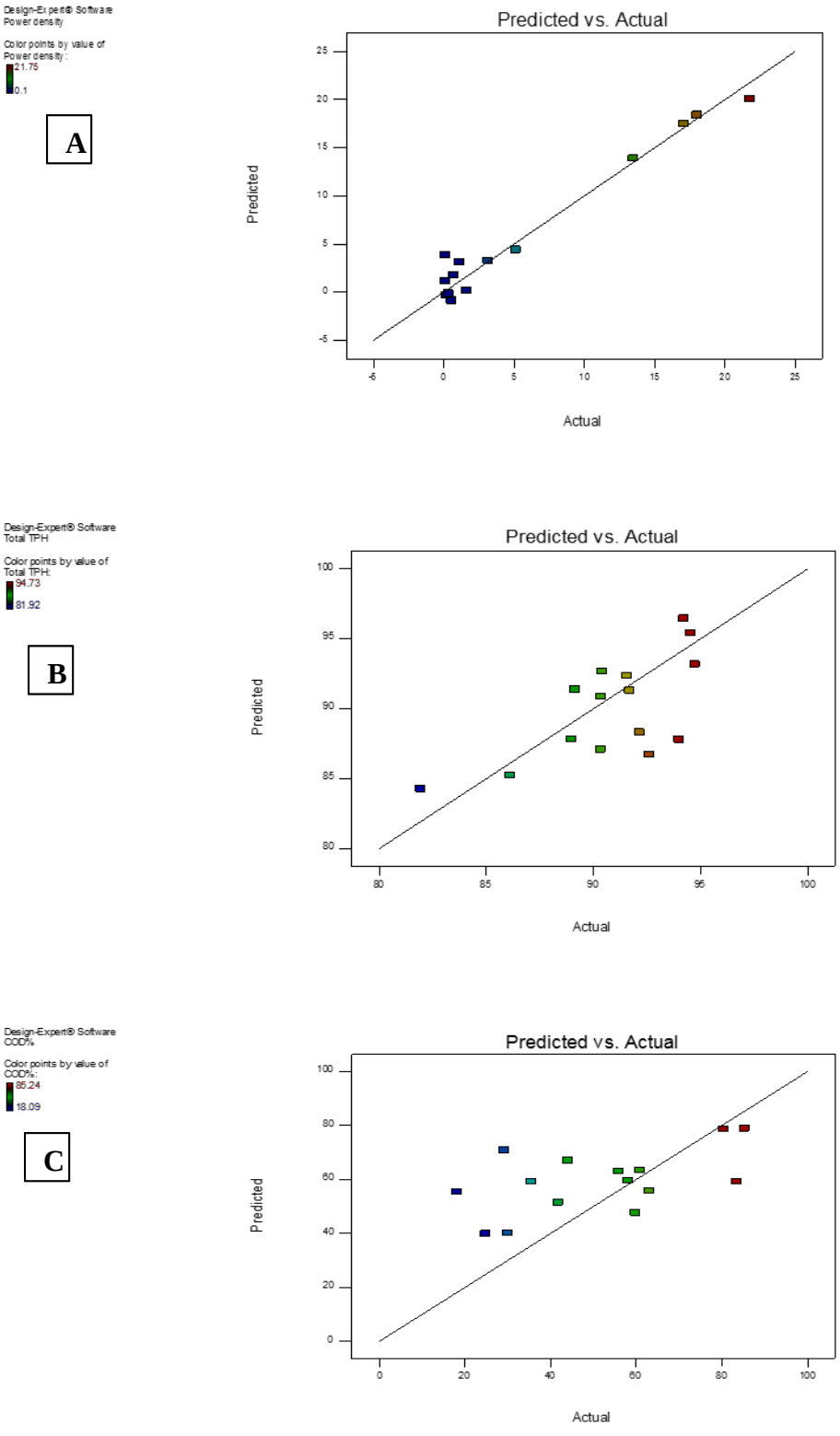


Figure 4.15: Predicted versus actual plots of different response factors namely; (A) Power density (B) COD removal and (C) TPH.

From Figure 4.16A, changes in external resistance had a huge impact on TPH removal relative to other factors such as salinity and redox mediator. A similar trend was observed in Figure 4.16B for response variable, power density. However, power density rose sharply with gradual increases in the external resistance applied across the MFCs up to the reference point. Thereafter, power density dropped consistently with further increases in both external resistance and redox mediator.

Increases in the concentration of redox mediators might be toxic to microbial community present in the MFC anode and very high external resistance could result in very low anode potential. At very low anode potential, below -350 mV (vs. NHE), very low energy is available for the microorganism for sustenance and electron transfer to the anode thus leading to drop in power output as observed in this study. Notably, there was also a positive correlation between power density and salinity. In Figure 4.16C, it can be observed that external resistance has no effect on COD removal efficiency but steadily decreases with increases in the concentration of the redox mediator and salinity (NaCl).

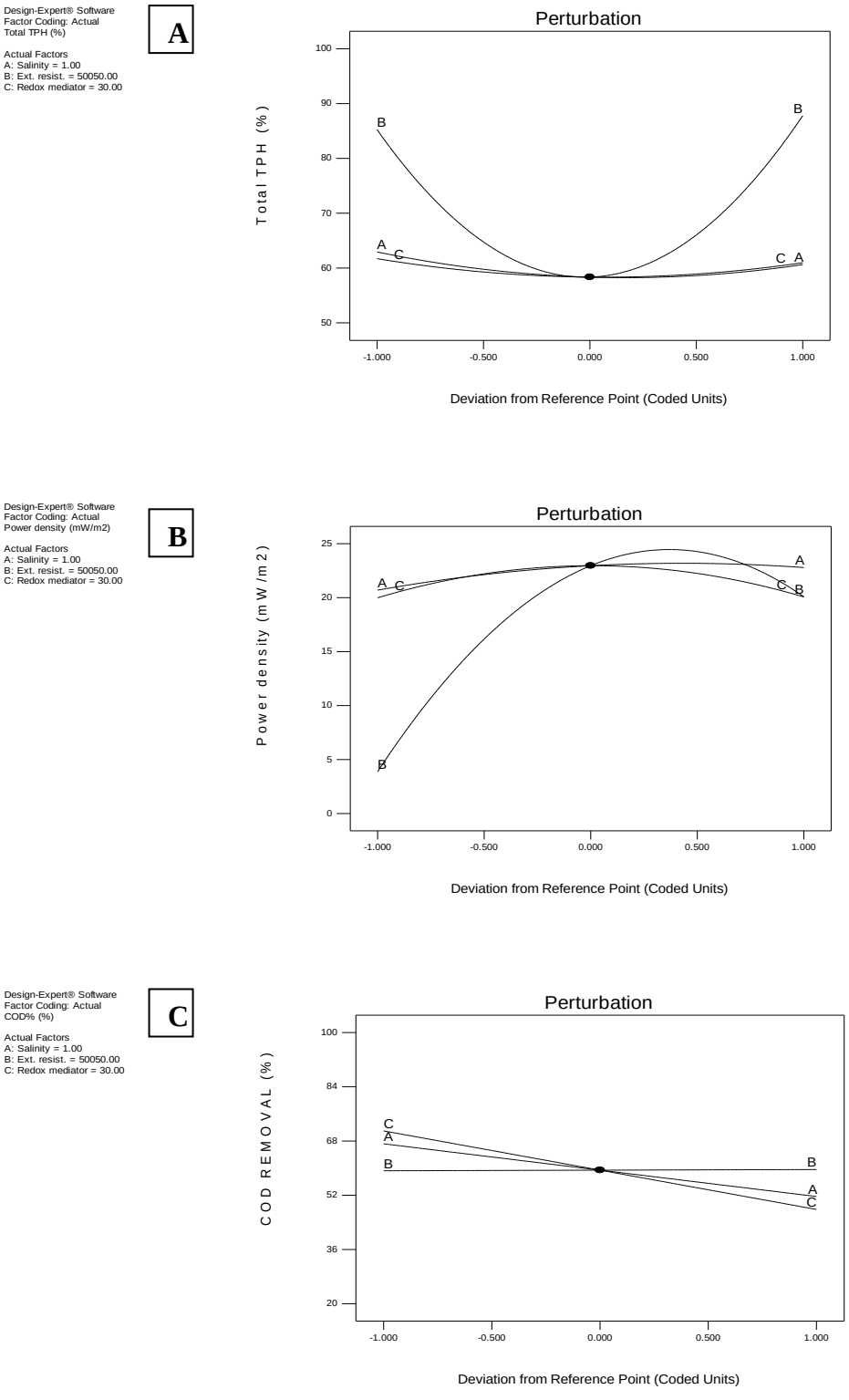


Figure 4.16: Perturbation plots indicating interaction effects of variables for each response factor; (A) TPH (Total petroleum hydrocarbons) (B) Power density and (C) COD removal. It should be noted that the chosen reference points for the factors; A (Salinity), B (External resistance), C (Redox mediator) are 1.0 % w/v NaCl, 50 kΩ and 30 µM Riboflavin respectively.

The following model (Equation (24)) was suggested for the response variable, power density (Appendix 3) using the statistical software, Design Expert 9.0.7. The fitted model is considered adequate if the F-test is significant ($P < 0.05$). The model F-value of 29.88 implies the model is significant. There is only a 0.01 % chance that a model F-value this large, could occur due to noise alone. The low probability value (<0.0001) indicates that the model is significant. The model was examined by the determination coefficient ($R^2 = 0.964$), which suggests that more than 96.42 % of the variance is attributable to the variables and indicated a high significance of the model. The response quadratic model is more accurate to describe influences of independent variables such as salinity, external resistance and redox mediator (riboflavin) studied when the obtained R^2 is closer to 1 (Liu and Tzeng, 1998; Zhang et al., 2012d).

Power density ($mWm^{-2)}$

$$= 22.96 + 1.05A + 8.10B + 0.039C + 0.99AB - 0.22AC - 0.26BC - 1.22A^2 - 10.98B^2 - 2.93C^2 \quad (24)$$

where A is salinity (NaCl concentration), B is external resistance and C is concentration of the redox mediator (riboflavin).

The Predicted R-Squared value of 0.803 is in reasonable agreement with the Adjusted R-Squared value of 0.932 (i.e. the difference is less than 0.2). Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 16.72 obtained in this research indicates an adequate signal. This model can be used to navigate the design space. The coefficient of variation (CV) is the ratio of the standard error of estimate to the mean value of the observed response (Mohajeri

et al., 2010). It is a measure of reproducibility of the model, generally a model can be considered reasonably reproducible if its CV is not greater than 10 %; the smaller CV is less dispersed than the variable with the larger CV (Reed et al., 2006). Hence, the low variation coefficient value (CV = 8.94 %) obtained indicates a high precision and reliability of the experiments. The coefficients of A, B, C and AB were positive, indicating that the contribution of those parameters was positive for power outputs, of which variable B (external resistance) had an obvious impact on power density among other variables. Whereas, variables AC, BC and B in this model, all had a negative coefficient indicating that, their contributions negatively affects the power production. Notably, it can be inferred that power outputs can be greatly enhanced at high external resistance and in the presence of increase concentration of salt, NaCl. These results are consistent with findings reported by others researchers (Lefebvre et al., 2012b; Keck et al., 2002).

Three-dimensional response plots for the effect of the interaction variables on power outputs were generated in this current study to determine the interaction among these two factors and their optimum concentration values as presented in Figure 4.17. In this kind of plot, responses were studied by taking two variables at a time while the other variable is kept at the '0' level (Avishek and Arun, 2008). Figure 4.17A showed the interaction of external resistance and salinity when maximising power outputs was set as the target. It could be seen that the response effect of salinity was less significant when compared with external resistance, while the interaction between them had obvious effects on power outputs.

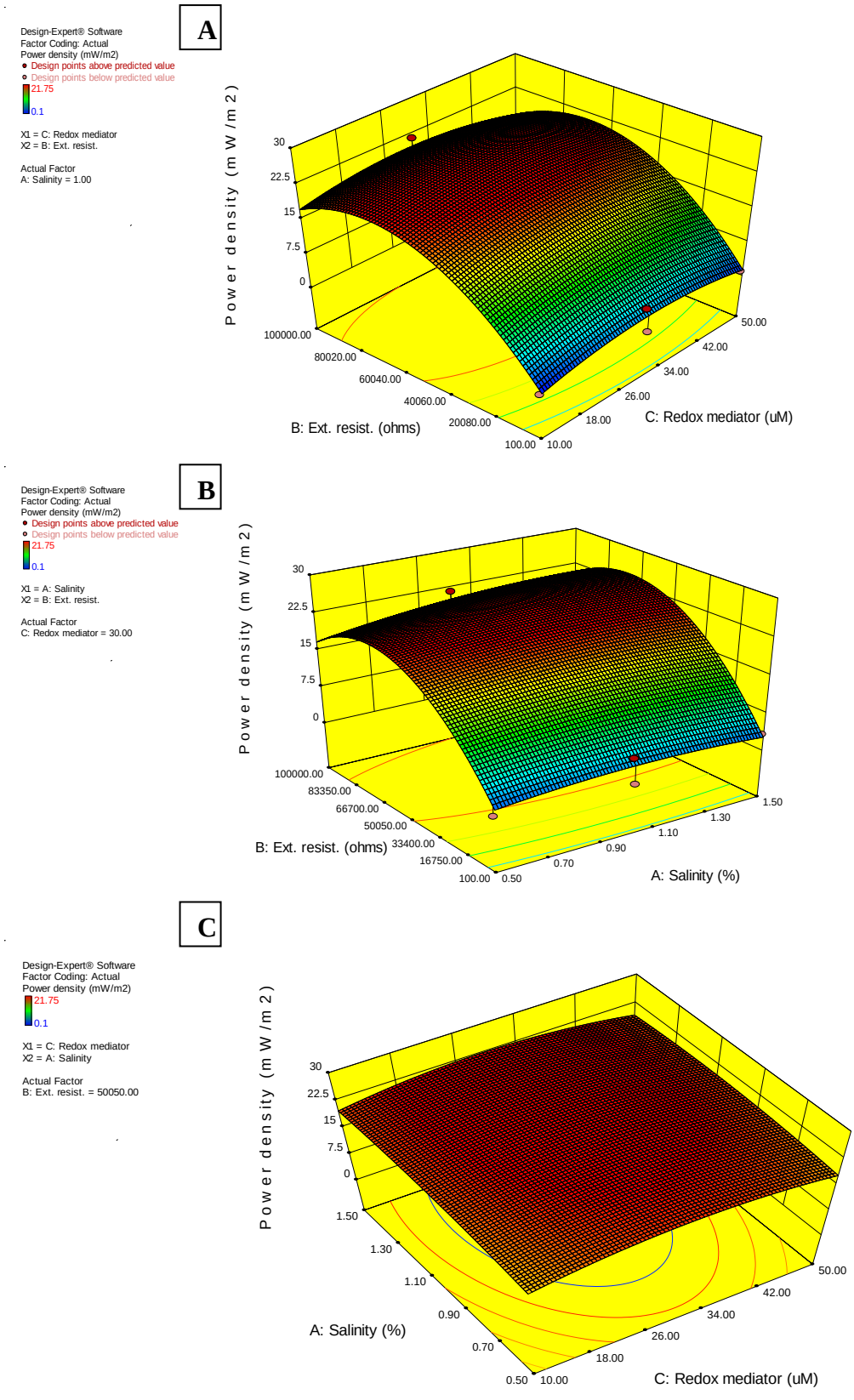


Figure 4.17: Response surface 3D plots for power density showing interaction effects of variables (A) External resistance and salinity (B) External resistance and redox mediator (C) Salinity and redox mediator.

The maximum power output (22.5 mWm^{-2}) was observed at high external resistance (83.35 kΩ) and a salinity of 1.3 % w/v NaCl. Maximum power density depends on both the total internal resistance of the MFC system and the external resistance applied across it (Zhang and Liu, 2010). At higher external resistances (60 kΩ to 100 kΩ), decrease in anode potential was observed, hence increasing the potential difference across the terminals with the possibility of a subsequent decrease in total internal resistance within the MFC.

The effect of the interaction of external resistance and redox mediator on power density is illustrated in Figure 4.17B. The three dimensional plots explains that the impact of external resistance is significantly more than redox mediator and that their interaction effect is negatively correlated. Notably, with further increase in concentration of redox mediator (greater than the optimal concentration of 30 µM), a slight decrease in power output occurred. This suggests that external resistance can be increased and that of redox mediator concentration has to be decreased for higher yields of power density. While at low external resistance, there was a marginal difference in power density with increasing concentration of the redox mediator.

The interaction effect of redox mediator and salinity has very little or no significant effect on power outputs as presented in Figure 4.17C. It is evident that change in the concentrations of both redox mediator and salinity has no significant effect on system performance in terms of power output. It also indicates that the concentrations of redox mediator and salt used has no obvious toxic effect on the microbial community present in the anode. These results are very well in agreement

with findings reported by other researchers (Don Santos et al., 2004; Lefebvre et al., 2012b; Menicucci et al., 2006).

Response surface 3D plots showing the interaction effects of variables (such as external resistance, redox mediator and salinity) on TPH removal efficiency are presented in Figure 4.18. From Figures 4.18A and 4.18B, changes in external resistance had a significant impact on power outputs compared to both redox mediator and salinity.

As previously discussed in this section, external resistance can determine, not only the anode redox potential, but also the selection of dominant species within the anode microbial community (Fernando et al., 2014a; Katuri et al., 2011; Jung and Regan, 2011). Depending on the applied external resistance, either high or low as in this particular study, we observed that TPH removal was optimised at very high (100 kΩ) and low (100 Ω) applied external resistance regardless of changes in the concentration of the redox mediator and salinity.

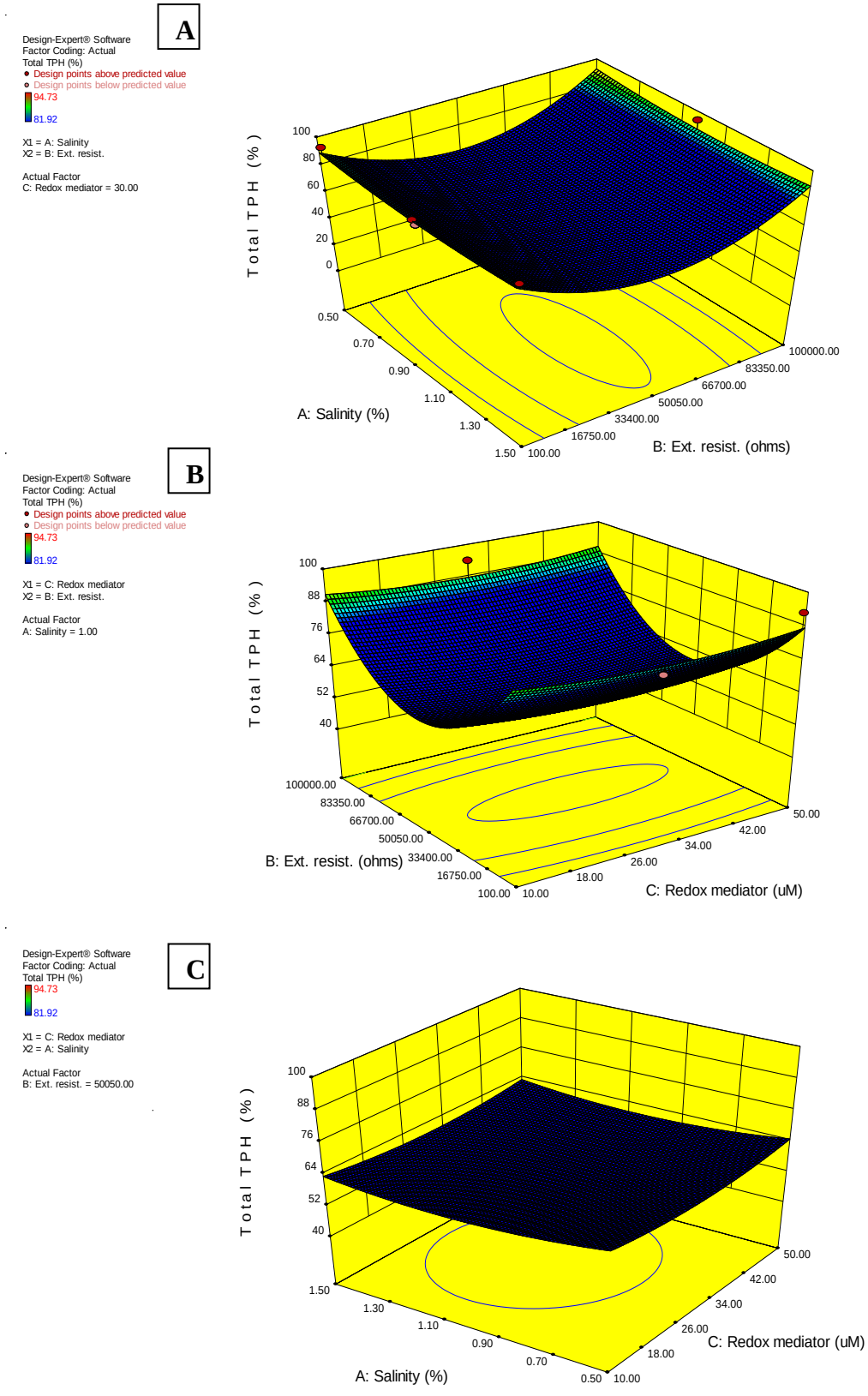


Figure 4.18: Response surface 3D plot for TPH removal indicating interaction effects of variables (A) External resistance and salinity (B) External resistance and redox mediator (C) Salinity and redox mediator.

This observation is in agreement with previous study reported by Katuri et al (2011). They demonstrated that change in applied external resistance of an MFC system led to a significant change in the anodic microbial population.

However, addition of redox mediator and salinity showed no correlation between them, although the TPH removal are slightly higher with increasing concentration of the redox mediator (Figure 4.18C). Notably, results revealed that external resistance had a significant impact on power density and TPH removal efficiency. Therefore, it implies that external resistance could be a potential tool for influencing degradation efficiency of petroleum hydrocarbon and optimising power production in MFCs.

4.2.11 Optimum process conditions and model validation

The optimum conditions for the independent process variables as predicted by the regression model, can be seen in Table 4.11. Numerical optimization based on desirability function was carried out. According to the optimum point predicted by the quadratic model, a maximum power production (24.89 mWm^{-2}) is achievable using the suggested levels. Moreover, to examine the adequacy of the model established, a triplicate validation experiments were performed at the predicted optimal conditions.

Results from the validation experiments are presented in Table 4.12. Notably, there was considerable agreement between the experimental and the predicted power outputs and the experimental values obtained were between the confidence levels (Table 4.11) of the predicted power density. Therefore, this confirmed that the quadratic model was suitable to predict the maximum power outputs obtainable

under optimised process condition. This further suggests that the model could be useful, within the given range of conditions, for verifying the reliability of the model.

Table 4.11: Optimisation result of statistical analysis and confidence levels.

External resistance (Ω)	**Salinity (w/v % NaCl)**	**Redox mediator (µM)**	**Power density (mWm^{-2})**	**Desirability**
69800	1.30	29.30	24.89	0.992
Response	**Prediction**	**SE Mean**	**95 % CI low**	**95 % CI high**
Power density (mWm^{-2})	24.887	1.239	21.985	28.504

Table 4.12: Confirmation experiment conditions and results using two-chambered MFC fed with petroleum hydrocarbons. Adapted microbial consortia was used as inoculum in the MFCs. Data presented below are three independent runs.

Run	**External resistance (Ω)**	**Salinity (w/v % NaCl)**	**Redox mediator (µM)**	**Power density (mWm^{-2})**
A	69800	1.30	29.30	23.217
B	69800	1.30	29.30	20.252
C	69800	1.30	29.30	24.047

The results from this study clearly showed that the independent parameters influence each other, and mutual interrelationship exists among process variables which could potentially exhibit significant impacts on the system or process performance. This is a unique feature of the statistical experimental design methodology compared to the traditional methods where simultaneous observation of two parameters has proven to be very difficult.

4.3 Concluding remarks

In this study, the performance of MFCs fed with a mixture of benzene and phenanthrene at operating conditions and the interaction effect of three selected operating conditions (external resistance, salinity and redox mediator) using RSM were investigated. A temperature of 40℃ was found optimal giving a maximum power density of 1.15 mWm^{-2}, a COD removal of 89.1 % and a degradation efficiency of 97.10 %; the optimal salinity was 1% w/v (as sodium chloride) giving a maximum power density of 1.06 mWm^{-2}, COD removal of 79.1 % and a degradation efficiency of 91.6 %. MFC performance in terms of electricity generation was enhanced 30 times when the redox mediator riboflavin (30μM) was added exogenously.

Triton X100 had lower toxic effect relative to Tween 80; only 20 CMC of Triton X100 was required in order to achieve maximum phenanthrene concentraion in aqueous phase. The addition of surfactant within the range tested increased apparent aqueous solubilty of phenanthrene but did not enhance degradation efficiency of phenanthrene when compared to a control; instead it rather slows down biodegradation rates. Optimal power density of 10.1 mWm^{-2} was obtained at an applied external resistance of 100 kΩ (10 fold higher than power obtained at 100 Ω) while maintaining a good degradation performance. Data from this study clearly indicates that varying external resistances had no significant effect on degradation efficiencies (for both phenanthrene and benzene) and COD removal efficiency but did impact significantly on electrochemical parameters such as voltage ouput, power density and internal resistance. High tolerance levels demonstrated by the adapted mixed culture to the petroleum hydrocarbons used in this study suggest their use in

the remediation of subsurface environments contaminated with high levels of benzene and phenanthrene concentrations.

Electrochemical performance in terms of power density and voltage output was increased by about 10 fold and 2.5 fold respectively compared to platinised cathode when potassium bromate was used as catholyte while good degradation performance was maintained at removal efficiency of 89.1 %. This study suggests the use of bromate in MFCs as catholytes or electron acceptors for significant improvement in power production in lieu of expensive and non-renewable metal catalyst.

RSM was performed to evaluate interaction among some selected operating factors and optimize the MFC system, with the target of maximizing power outputs. The results from this study clearly showed that the independent parameters influenced each other and external resistance however, significantly influenced system performance, especially in term of power outputs. Results indicated that RSM was effective to evaluate and optimize MFC system performance, thus providing guidance to the practical application of the system in the future. This could make MFC technology more attractive for use as preferred remediation strategy in treatment of oil-contaminated groundwater or refinery effluents, considering added economic value in terms of energy recovery.

CHAPTER 5

MFC APPLICATIONS TO AQUEOUS SYSTEMS: Treatment of petroleum hydrocarbons in a continuous operation using two different tubular MFC designs.

5.1 Chapter overview

For practical applications, MFCs would have to be effective, efficient, and robust and applied *in situ.* BES systems need to be designed uniquely and tested for their performance before its possible to deploy them for either *in situ* or *ex situ* applications. In wastewater treatment technological applications, hydraulic retention time (HRT) is an important design parameter which has significant influence on system design, operational/investment cost, process efficiency and energy requirement (Kuscu and Sponza, 2009; Shariati et al., 2011). In general, lower HRTs are desirable from economic viewpoints because higher HRTs will lead to greater investment costs. Varying HRT could also have a significant effect on power outputs and removal efficiency of the MFC system (Zhang et al., 2012d).

There are several studies in which the effect of HRT on degradation/COD removal efficiency and power density have been examined (Zhang et al., 2012d; Jayashree et al., 2014; Feng et al., 2014; Akman 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). However, in this current study, a tubular MFC design was employed to study the effect of HRTs on MFC performance; this MFC design has never been reported in the literature.

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/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 across different conditions may be added an advantage of MFC technology over conventional anaerobic technologies aside from energy recovery. Therefore, it is necessary to evaluate the effect of substrate availability at very low or high concentrations on MFC performance.

In this regards, this study has investigated the performance of a tubular MFC, operated in a continuous mode at different hydraulic retention times, HRT at ambient temperatures. Other important aspects of operation such as toxicity assessment of MFC and the reactor's response to low and high substrate levels conditions were also investigated.

5.2 Results and discussion

5.2.1 Treatment of petroleum hydrocarbons using a two chambered-tubular MFCs

5.2.1.1 Effect of different HRTs on power generation and degradation performance of the 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 (Figures 5.1 and 5.2). COD reduction was observed (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 (i.e. about 20-30 %) lower than that of the open circuit control (i.e. analogous to a conventional anaerobic process) as depicted in Figure 5.1 A.

As indicated in Figure 5.1B, the 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 (HRT of 2 d).

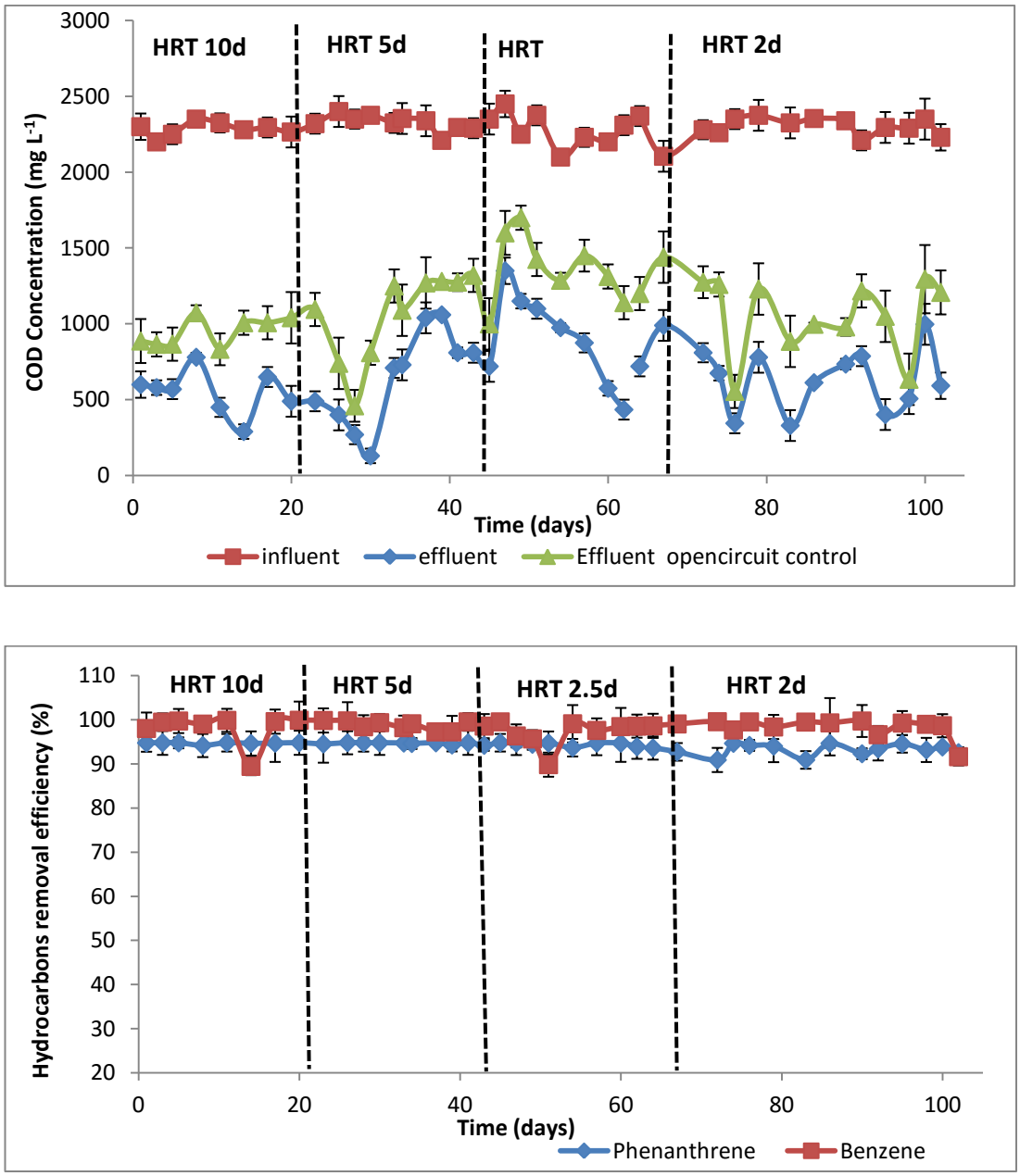


Figure 5.1: (A) COD removal and (B) Hydrocarbons removal efficiencies at various HRT regimes over a period of 110 days of continuous MFC operation at ambient temperatures (14-25 ℃) using adapted microbial consortia. Values are means of duplicate experiments ± SD.

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.

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 (10-25 ℃) 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.

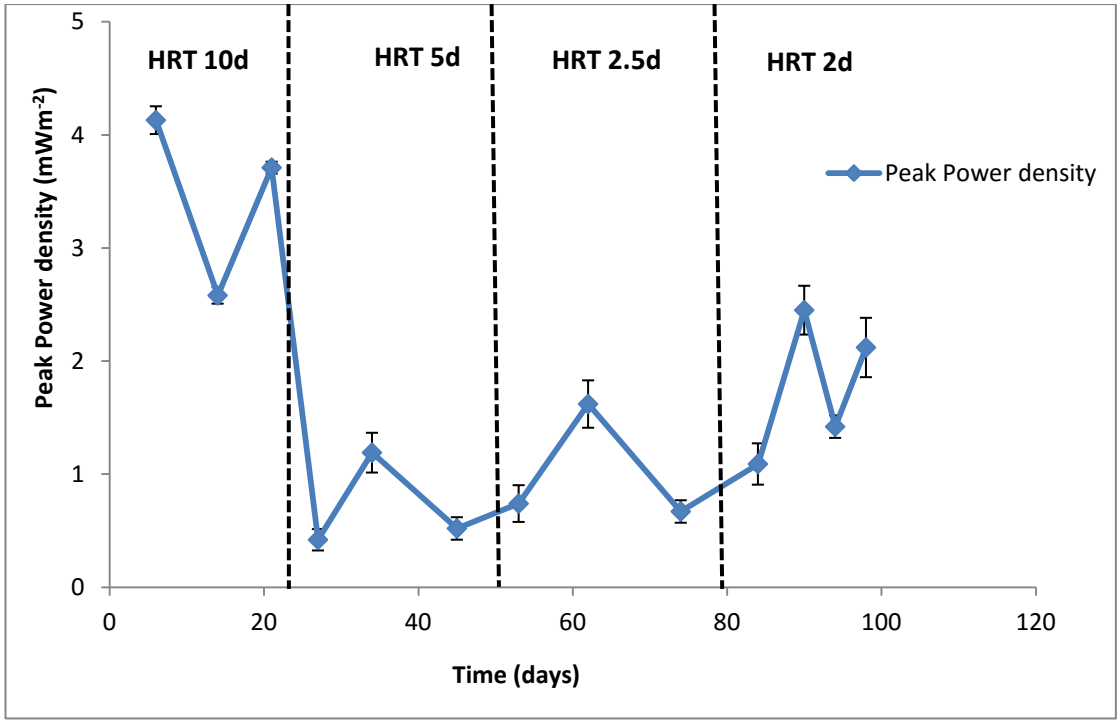


Figure 5.2: Profile of peak power densities produced at different HRTs in a tubular MFC over 100 days continuous operation at ambient temperatures (10-25 ℃) using adapted microbial consortia. Values are means of duplicate experiments ± SD.

In order to understand the influence of HRT on the power output and COD removal, the MFCs fed with petroleum hydrocarbons were operated continuously under four different HRTs (2, 2.5, 5, and 10 d). Average total COD removal efficiencies decreased significantly (at p= 0.01) from 74 % to 57 % with decreasing HRT (Figure 5.3). The minimum average total COD removal efficiency (57 %) was observed at HRT 2.5 d as shown in Figure 5.3. There was sharp decrease in average power outputs as HRT from change from 10 to 5 d and thereafter a steady rise in power production was observed with stepwise decrease in HRT.

Open circuit control indicated an 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 a 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 have caused a shock 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 increased, due to change of seasons and microbial adaption, as steady recovery in power outputs was observed which corresponded with a slight increase in COD removal efficiency.

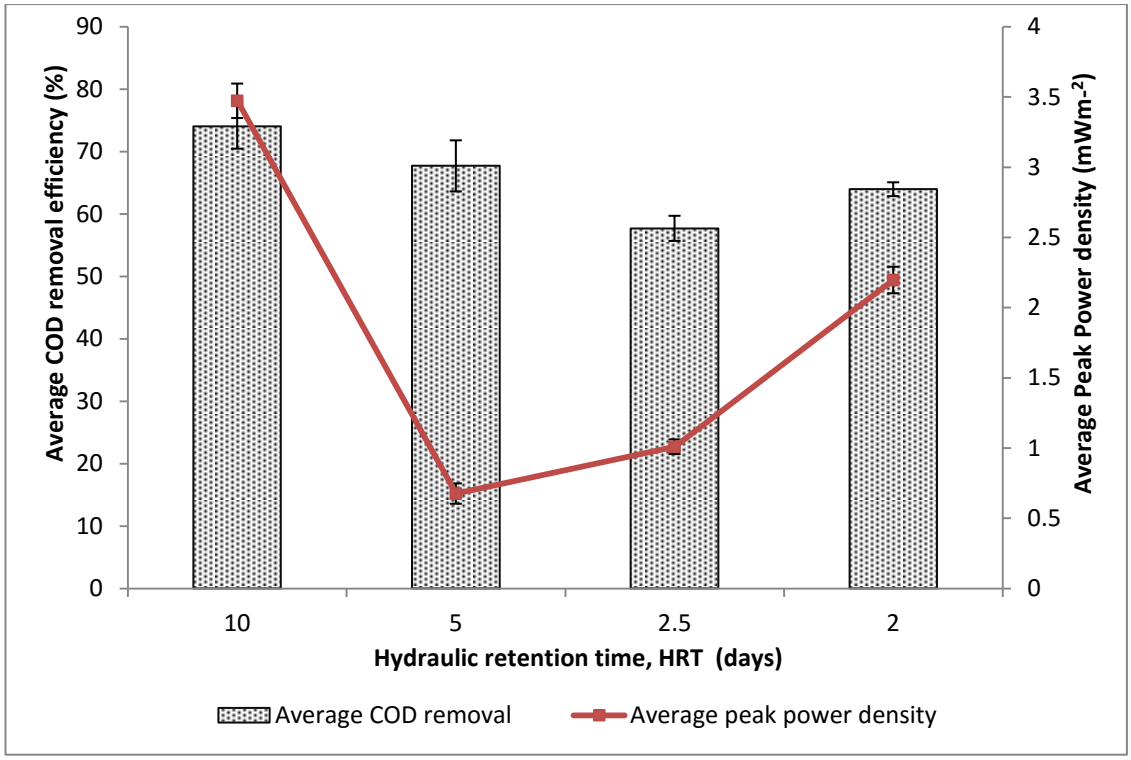


Figure 5.3: COD % removal and peak power density over the test period in MFCs at different HRTs in the tubular MFC at ambient temperatures (10-25℃) using adapted microbial consortia. Error bars ± SD are based on duplicate measurements.

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 decreasing HRT. This suggests the potential practical applications 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).

5.2.1.2 Simultaneous bromate removal during MFC operation.

In the cathode chamber of the MFC reactor, bromate was used as terminal electron acceptor in lieu of a platinised cathode. From Figure 5.4, 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 might be that the increase in residence time allowed more electrochemically transfer of electrons (resulting from the anodic oxidation in the anode) and creates the reduction of bromate ions to bromide ions. Bromate ion is well known toxic pollutant which is classified as carcinogenic in nature (especially to humans) by International Agency for Research on Cancer (IARC). The WHO standard for drinking water is 0.01 mg L^{-1} for drinking water and the EU maximum limit for bromate in natural mineral and spring waters is 3 µg L^{-1} (Zhao et al., 2012). The conversion of bromate ions through electrochemical process (as used in this study) to non-toxic 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. In other words, 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).

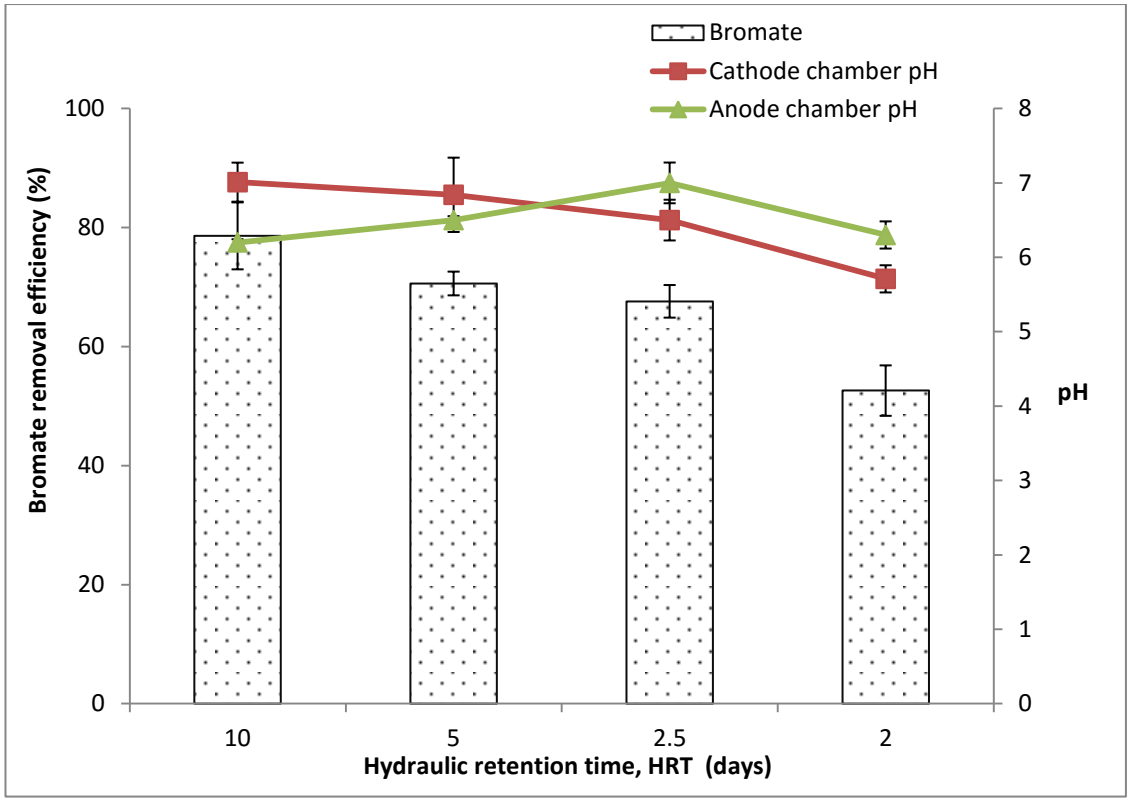


Figure 5.4: Bromate removal performances and changes in pH in the tubular MFC reactor over 110 days operation at ambient temperatures (10-25℃) using adapted microbial consortia. Error bars ± SD are based on duplicate measurements.

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 ..... (25)

$HOBr + H^+ + 2e^- \rightleftharpoons Br^- + H_20$ 1.341 V vs SHE ..... (26)

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 generally have difficulty approaching the cathode because of electrostatic repulsive force (Zhao et al., 2012). During continuous MFC operation, the bromate in the cathode chamber is reduced to bromide ions which can be either adsorbed on the cathode (a carbon electrode) or released as bromine gas due to the reaction between two bromide ions. This depends 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 decreased performance in bromate removal. Slight increase in anode pH, which probably indicates the formation of acidic metabolites resulting from microbial anaerobic oxidation processes 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 the fact that the high cost of platinum and possible catalyst poisoning or wash off (especially in environmental matrices) has profoundly limited its use in large scale MFC operations (You et al., 2006b; Rabaey et al., 2004), bromate contaminated water could be a potential replacement for Pt cathodes in MFCs as previously demonstrated (chapter 4). 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 would be ineffective due to the lack of air or oxygen.

5.2.1.3 Effect of low and high substrate levels conditions on MFC operation

For a continuous bioreactor to be deployed for potential *in situ* application 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 a range of nutrient & substrate 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 d) at low and high substrate levels conditions under ambient temperature (14-22℃).

Good MFC performance (in terms of COD removal effciency) was obtained with between 65 and 95 % removal in both nutrient conditions (Figure 5.5). Maximum power density of 0.76 mWm^{-2} was obtained under high substrate level conditions. 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 in low substrate condition. The concentration of petroleum hydrocarbons after treatment in low (oligotrophic) substrate conditions were below the instrument's dectection limit therefore, data on their degradation efficiencies 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 at different operating conditions (Fernando et al., 2014b; Senturk et al., 2012; Karim and Gupta, 2006; Jayashree et al., 2014). However, at very low COD/polluant levels, substrate concentration can become a rate limiting factor to microbial degradation.

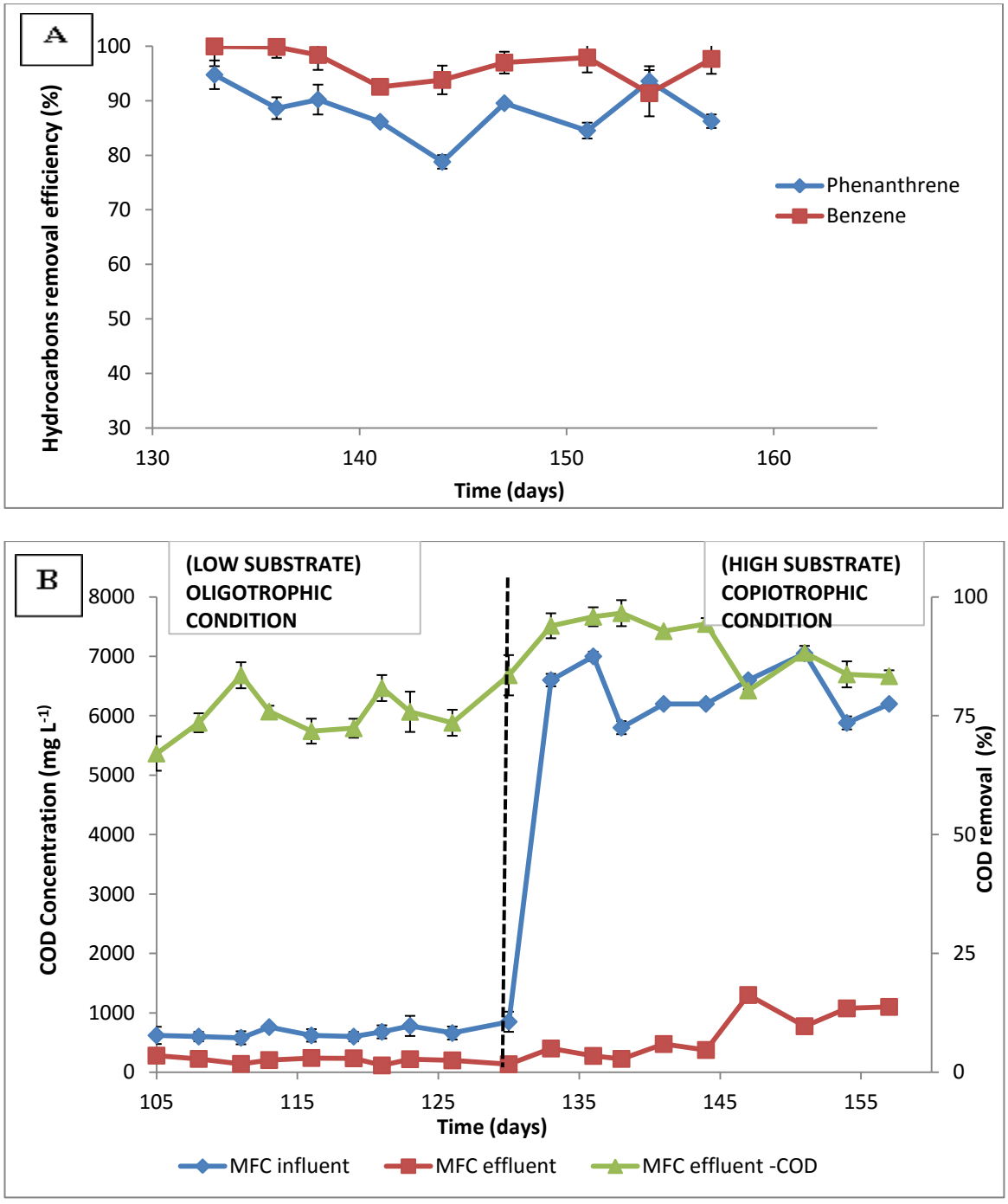


Figure 5.5: (A) Degradation performance of the tubular MFC at high substrate condition (B) COD removal at low and high substrate conditions operated in a continuous mode at HRT of 10 d. Error bars ± SD are based on duplicate measurements.

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.

However, in this MFC reactor, in low substrate conditions, power production was extremely low indicating very little or dormant microbial acitivity taking place resulting from low substrate concentrations. Another possible reason for this observation could be that the biofilms formed on the anode adapted to this harsh condition by allowing the microbial cell 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. This speculation was further supported by fast recovery in overall MFC performance as the MFC was switched to high substrate conditions.

Findings of the 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* application in contaminated groundwater treatments due to its effectiveness and robustness even at extreme conditions as demonstrated in this study.

5.2.1.4 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 increased in HRT from 2 to 10 d. The MFC effluents at both HRTs were generally less toxic than the influent as shown in Figure 5.6.

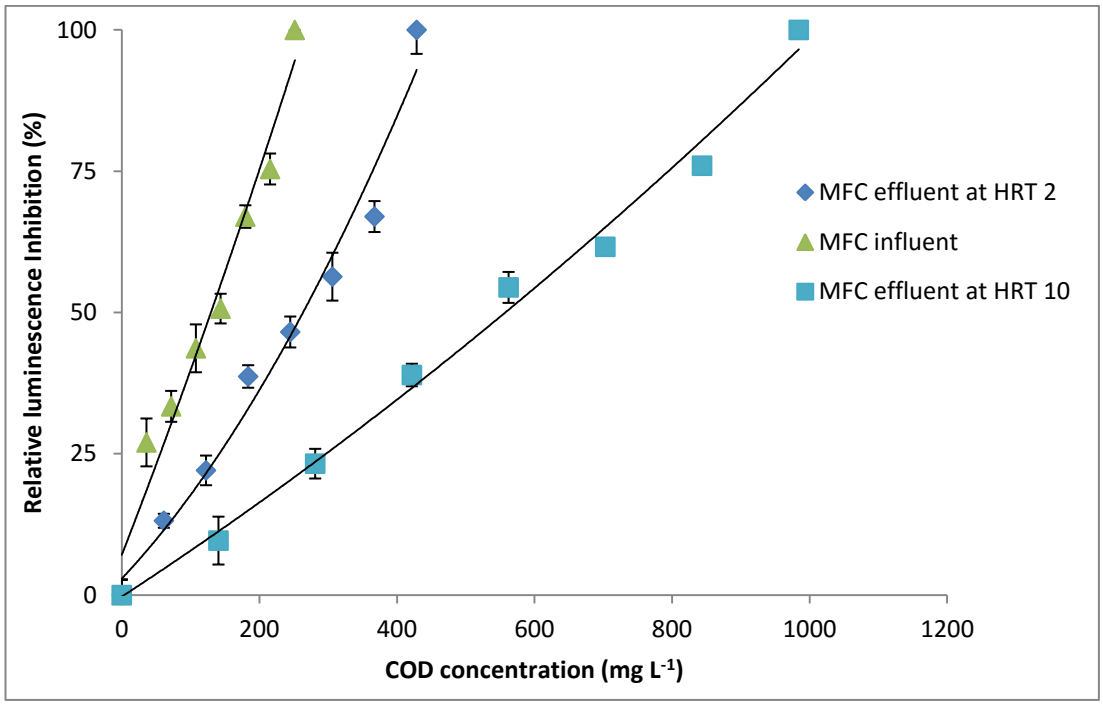


Figure 5.6: Bioluminescence based toxicity determinations of MFC effluent and influent at HRTs 10 and 2 days using bioluminescent marine bacteria, *V.fischeri*. Values are means of duplicate experiments ± SD.

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 within the reactor leading to greater breakdown of any 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 forms 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).

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 agree with the few other 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 above in some other sections (section 5.2.1.1 and 5.2.1.3) of this study further demonstrate the effectiveness of this tubular MFC in the *in situ* treatment of petroleum hydrocarbons especially for deep aquifers with contaminated groundwater.

5.2.2 Effect of HRT on MFC performance using a single chamber-tubular MFC fed with petroleum hydrocarbon-containing wastewater.

5.2.2.1 Performance of an MFC at different HRTs and under high substrate condition.

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 Figure 5.7A. A consistent drop in COD levels (1000 to 200 mg L^{-1}) was observed as HRT was changed from 10 to 30 h. which was significantly higher compared to the MFC open circuit control over the incubation period (Figure 5.7). 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 (Figure 5.8). 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, that could possibly determines the content of effluent substrate and power generation in the MFC. As previously discussed in section 5.2.1.1, high HRT infers more contact time between the microbes and the substrate within the MFC reactor which may have been 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).

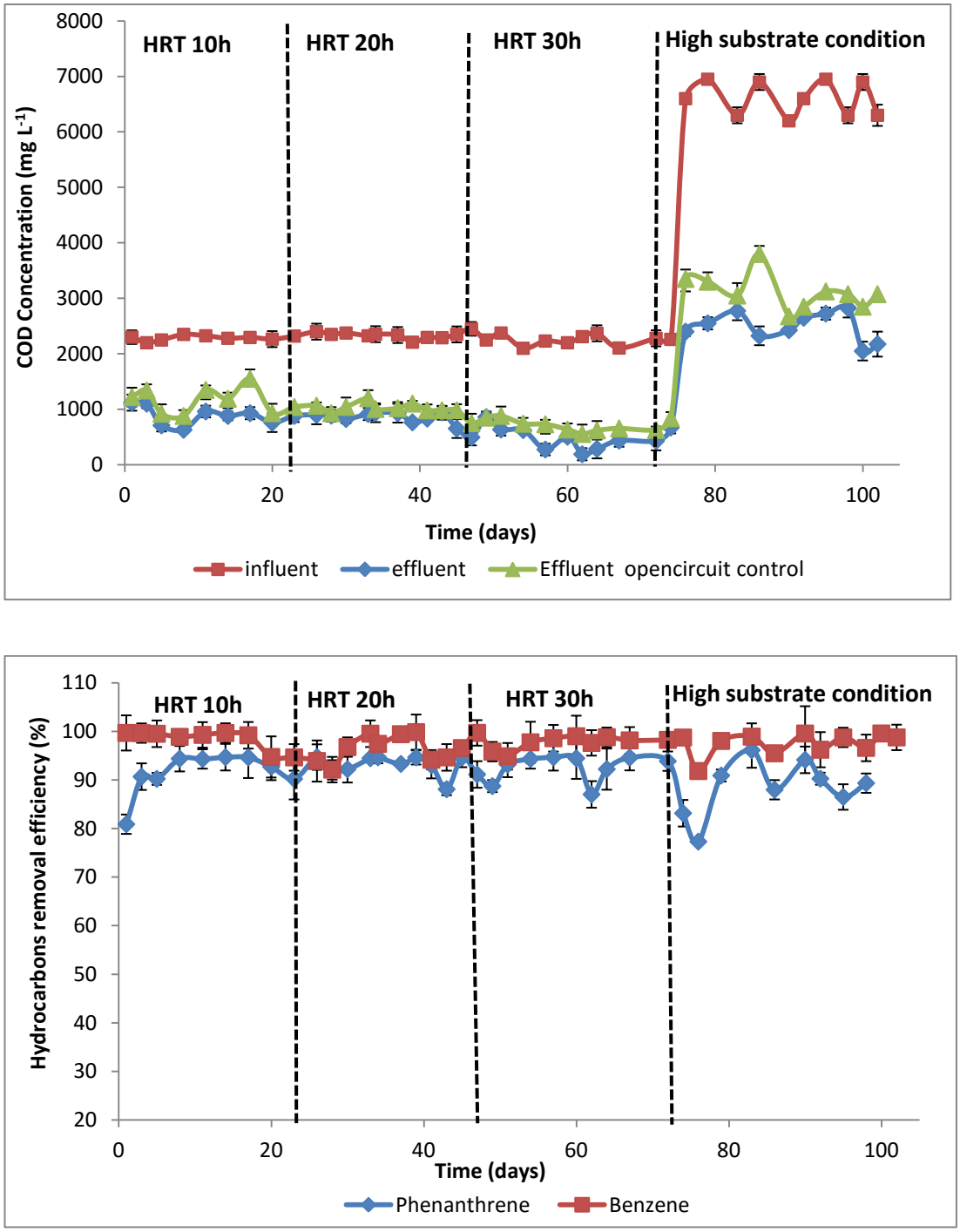


Figure 5.7: COD removal and hydrocarbons removal efficiencies at different HRTs and high substrate level condition at ambient temperatures (15-25℃) using adapted microbial consortia. Error bars ± SD are based on duplicate measurements..

As indicated in Figure 5.8, high HRT favoured increasing power generation in an air - breathing tubular MFC and the HRT of 30 h was found to be optimal for good MFC performance. The results of this study were similar to those of 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 the 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 increased at longer HRTs as observed in this study and other previous studies. This could be due to the longer contact time between biofilms and organic matter in the anolyte, which would benefit biofilm uptake, substrate degradation, and facilitate the transfer generated electrons onto anode surfaces (Sharma and Li, 2010).

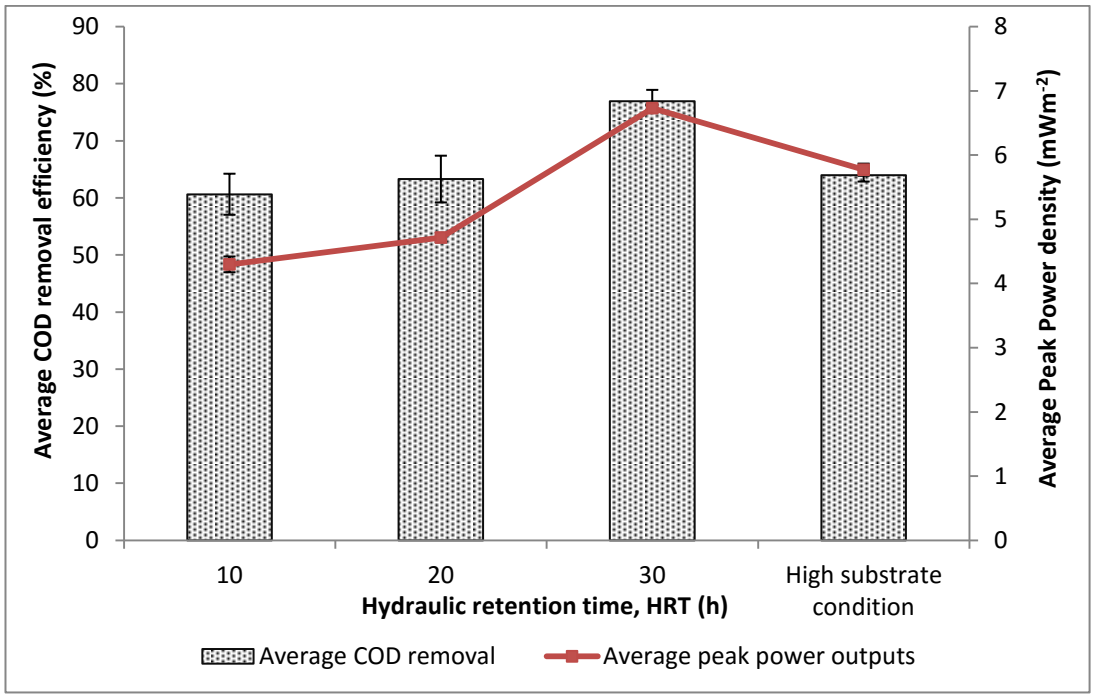


Figure 5.8: Average COD removal efficiencies and peak power densities generated as a function of time at different treatment conditions in a tubular MFC system over 100 days period of continuous operation using adapted microbial consortia. Values are means of duplicate experiments ± SD.

The results of this study implied that pollutant removal and power recovery could be maximized by increasing HRT to a preferred value. It provides the possibility of treating high strength organic wastewater in future applications.

In copiotrophic conditions (high organic loading), a 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 can maintain its performance (in terms of COD removal) without any process inhibition (Fernando et al., 2014b; 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 (Figures 5.7 and 5.8). 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.

5.2.2.2 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). Figure 5.9 shows the inhibition

of the bioluminescent marine bacteria, *V. fischeri*, due to the direct contact with liquid samples of MFC effluents taken from both MFC reactors running at different operating conditions (i.e. MFCs running at HRT 30h and high substrate conditions). Bioluminescence based acute toxicity assays conducted using *V. fischeri* indicated reductions of 61 % and 42 % in toxicity of effluents after treatment at HRT 30 h and high substrate (operated at HRT 30h) condition respectively. The observed decrease in toxicity (as indicated by a drop in bioluminescence inhibition of test microorganism) may be attributed to the transformation of the parent pollutants to less harmful metabolites. 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).

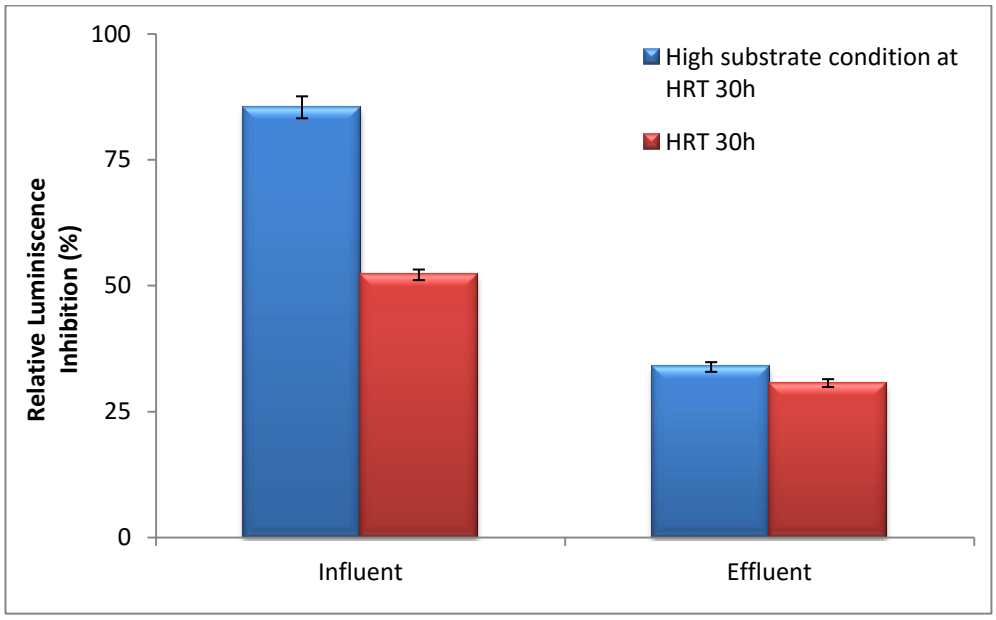


Figure 5.9: Toxicity levels of MFC effluents at HRT 30h and high substrate condition using adapted microbial consortia as inoculum source. Values are means of duplicate experiments ± SD.

5.3 Concluding remarks

In the first part of this chapter, the effect of change in HRTs on the performance of a two-chambered-tubular MFC was assessed. This study clearly demonstrated that HRT significantly influenced MFC performance with HRT of 10 d identified as the suitable operating parameter for optimum system performance (degradation efficiency, 96 %, COD removal efficiency, 74 % and peak power density, 3.4 mWm^{-2}). MFCs could also withstand extreme substrate level conditions. This suggests the application of MFCs in the simultaneous removal of petroleum hydrocarbons and bromate (with concomitant biogenic electricity generation) in anoxic environments, especially deep groundwater reservoirs. MFC technology could possibly be a substitute for the more expensive conventional technologies such as permeable reactive barrier (PRB) and electroremediation which are currently employed in the remediation of hydrocarbon pollutants in subsurface environment, especially at depth higher than 300 m where existing technology, PRB systems can not be deployed due to its technology limitations.

In another study, the performance of a tubular MFC operated in a continuous mode at different HRTs (10-30 h) and high substrate (copiotrophic) conditions under ambient temperatures (20-25℃) was investigated. 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. In copiotrophic conditions, a 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 while maintaining good petroleum hydrocarbon efficiencies. This study suggests the potential use of MFC technology for possible *ex situ* hydrocarbon-contaminated groundwater treatment or refinery effluents clean-up even at extreme (high contaminantor substrate levels) condition.

CHAPTER 6:

MFC APPLICATIONS TO SOIL SYSTEMS: Enhanced bioelectro-chemical remediation of phenanthrene-polluted soil

6.1 Chapter overview

Soil microbial fuel cells (sMFC) are a new technology for organic contaminated soil remediation with simultaneous electricity generation without need for *in situ* addition of any electron donor or electron acceptor into the soil or subsurface environments. Morris and Jin, 2012; Zhang et al., 2010a). Electrodes in MFCs can provide a low-cost and low-maintenance pathway for the passage of electrons from the anode to the cathode (which does not corrode over long-term deployment) thus stimulating the anaerobic degradation of organic pollutants (Morris and Jin 2008; Zhang et al., 2010a; Wang et al., 2012b). In particular, soil MFCs can stimulate and enhance hydrocarbon degradation accompanied with energy production without requiring any chemical addition or energy input, hence making operational costs substantially lower than other remedial methods. Moreover, electricity production during MFC operations can serve as a real-time bioremediation indicator and also power wireless sensors for remote online monitoring (Donovan et al., 2011).

Few studies on bioelectrochemically-assisted soil/sediment bioremediation have been reported for phenol (Huang et al., 2011), BTEX compounds (Zhang et al., 2010a), petroleum hydrocarbons (Morris and Jin, 2012) and PAHs (Wang et al., 2012b). Morris and Jin (2012) observed that of the biodegradation rate of TPH in sediment increased by 12 times compared to that of the background. Wang et al (2012b) demonstrated that a U-tube MFC increased TPH removal by 120 %, from 6.9 % to 15.2 %, for water saturated saline soil close to the anode (<1 cm), but the MFC performance decreased with larger distances from the anode or lower water content. Despite the previous studies on the 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 other cathodes electron acceptors such as bromate with petroleum hydrocarbon degradation using a tubular soil MFC bioreactor design. Bromate has previously been demonstrated above (section 4.2.5) 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., 2012; Bao et al., 1999).

The radius of influence (ROI), which is a distance from the anode where biodegradation continues to be enhanced, could to a large extent determine the full scale implementation of the newly design tubular MFC for *in situ* bioremediation of oil-contaminated soil. The ROI can be largely influenced by MFC architecture and electrode materials, as well as physiochemical and biological properties of the soil and contaminants.

In this study, new designs of column-type MFCs were developed for enhanced biodegradation of phenanthrene (a model PAH compound) in soil, as determined by changes in 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. Change of pH and electrical conductivity of the soils were also monitored in order to explore their impact on phenanthrene biodegradation. Results from this study were expected to provide important information for the field application of MFC technology for enhanced bioremediation of petroleum contaminated soils.

6.2 Results and Discussion

6.2.1 Pollutants removal and ROI determination during MFC operation

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. The temporal and spatial distributions of phenanthrene removal during the 60 days MFC operation are shown in Figures 6.1 and 6.2. At day 10, phenanthrene removal from soil at 2 cm from the anodes outer surface were 55 % and 50 % for MFC+S and MFC-S reactors respectively, with an increase in degradation efficiency of 120-293 % higher than the non-MFC reactors (E-S and F+S) having 14 % and 24 % phenanthrene removal respectively (Figure 6.1). 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 (2010a) and Lu et al (2014b) where similar observations reported were linked to hydrocarbon adsorption unto the electrode's surface.

Phenanthrene degradation rates appeared to decline 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 6.2), indicates a smaller radius of influence 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 transfer towards the electrode resulting into further increase of the ROI.

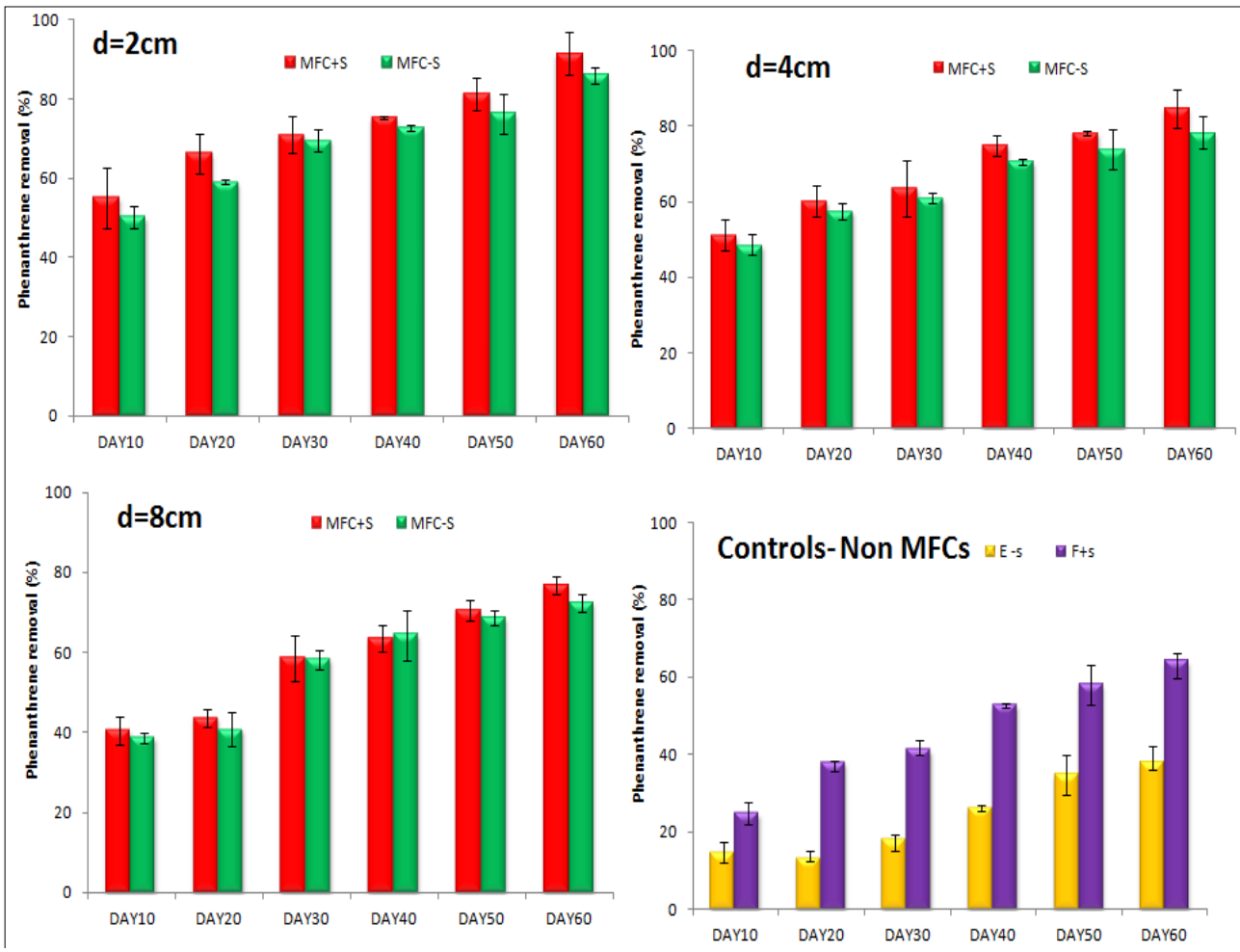


Figure 6.1: Phenanthrene removal in soil at distance of 2 - 8 cm from the anode during the operating period in MFCs with MFC only (MFC-S) and MFC with surfactant (Tween 80, 250 mg L^{-1}) amendment (MFC+S) using indigenous soil microorganism as inoculum source. Controls were prepared in the same reactors 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 rates in active MFCs were consistently higher than those in the control reactors, indicating the positive

influence of MFCs on degradation regardless of their distances from the anode's outer surface.

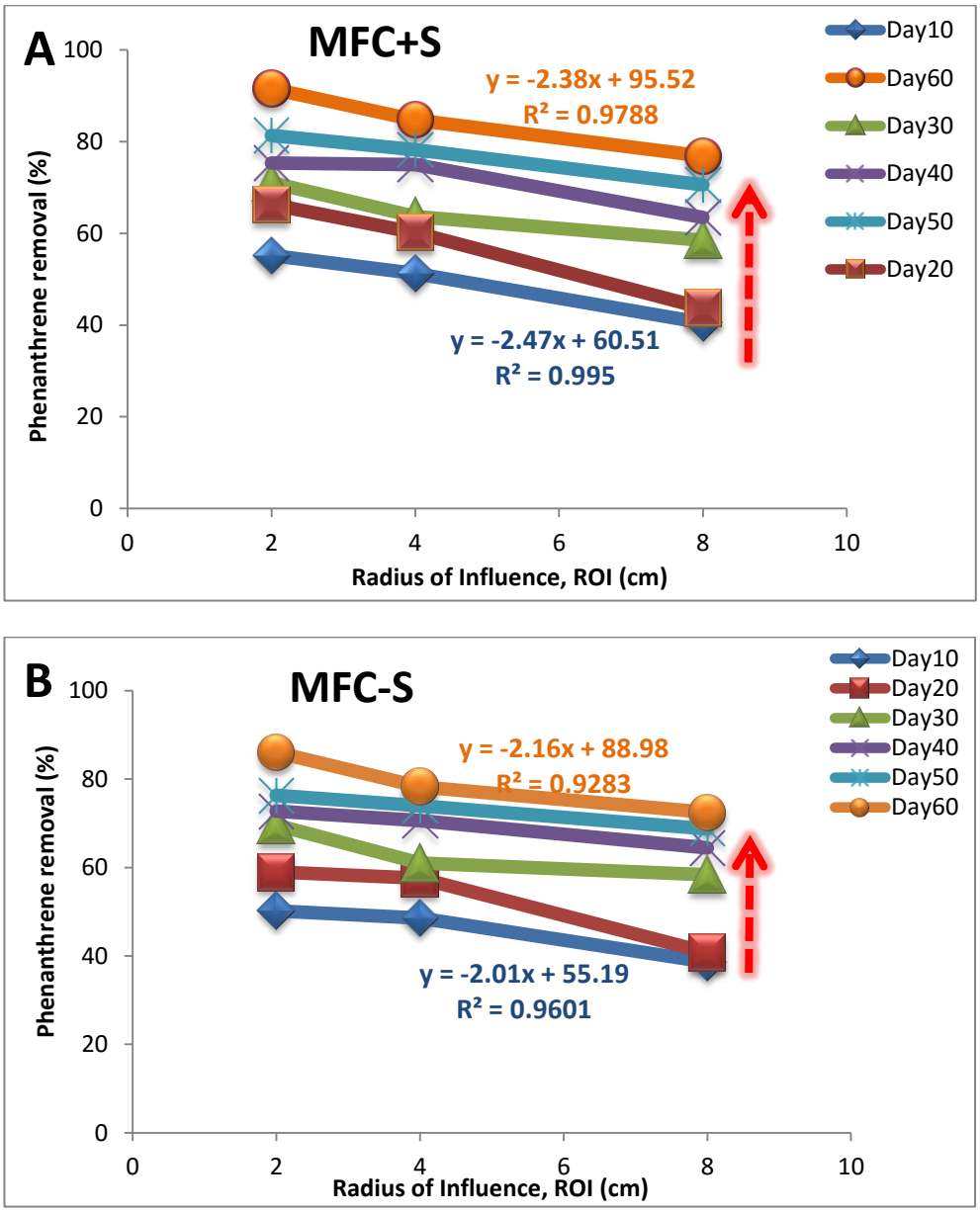


Figure 6.2: Relationship between phenanthrene removal and the radial distance from the MFC anodes at different sampling times. Dashed arrows indicate that maximum ROIs were expanding as reflected by the flattening of the slopes.

Phenanthrene removal rates continued to rise with time and reached 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 approximately the same for all radial distances from the anodes (Figure 6.1). There was a

statistically significant difference (at p=0.001) between 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. Consequentially, this may have led to improved mass transfer to the electrode and supported faster degradation rates.

The dose of surfactant used in this study (250 mg L^{-1}) appears to increase the surfactant sorption onto soil, which may have resulted in increased phenanthrene partitioning onto soil particles. The result of this study is in agreement with previous studies conducted by Lu et al (2014b) on the influence of ROI on enhanced bioremediation of petroleum-hydrocarbon contaminated soil in MFCs using two different low-cost electrodes - biochar and graphite electrodes.

In this study, the actual measurement of the radius of influence (ROI) for the soil MFCs was limited to the configuration of the lab reactor; however, theoretical ROIs could be extrapolated based on the data. The linear regression equations derived for active MFCs at day 10 and 60 can be used to descriptively explain and predict the expansion of ROIs with time in this saturated soil condition (Figure 6.2 A and 6.2 B). 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. From day 10 to day 60, the estimated maximum ROI increased 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 (2014b) demonstrated a further increase in maximum ROI at longer periods (about 120 days) of MFC operation under similar operating conditions corroborate 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 so 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.

The phenanthrene concentration in the aqueous phase of the soil MFCs was relatively constant across both active 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 6.3B). The insignificant change in porewater phenanthrene concentration (especially in MFC+S and MFC-S reactors) might be due to the dynamic balance in the phenanthrene partioning between the soil-phase and the aqueous phase. There presumed to be a possible balance between the phenanthrene degradation and desorption rates in the MFC.

Figures 6.1 and 6.3A clearly demonstrate that phenanthrene degradation close to the electrode was significantly enhanced compared with 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 contaminant adsorbed by the electrodes were biodegraded by anodic microbial respiration rather than abiotic adsorption and that the adsorption process merely enhanced faster biodegradation rates.

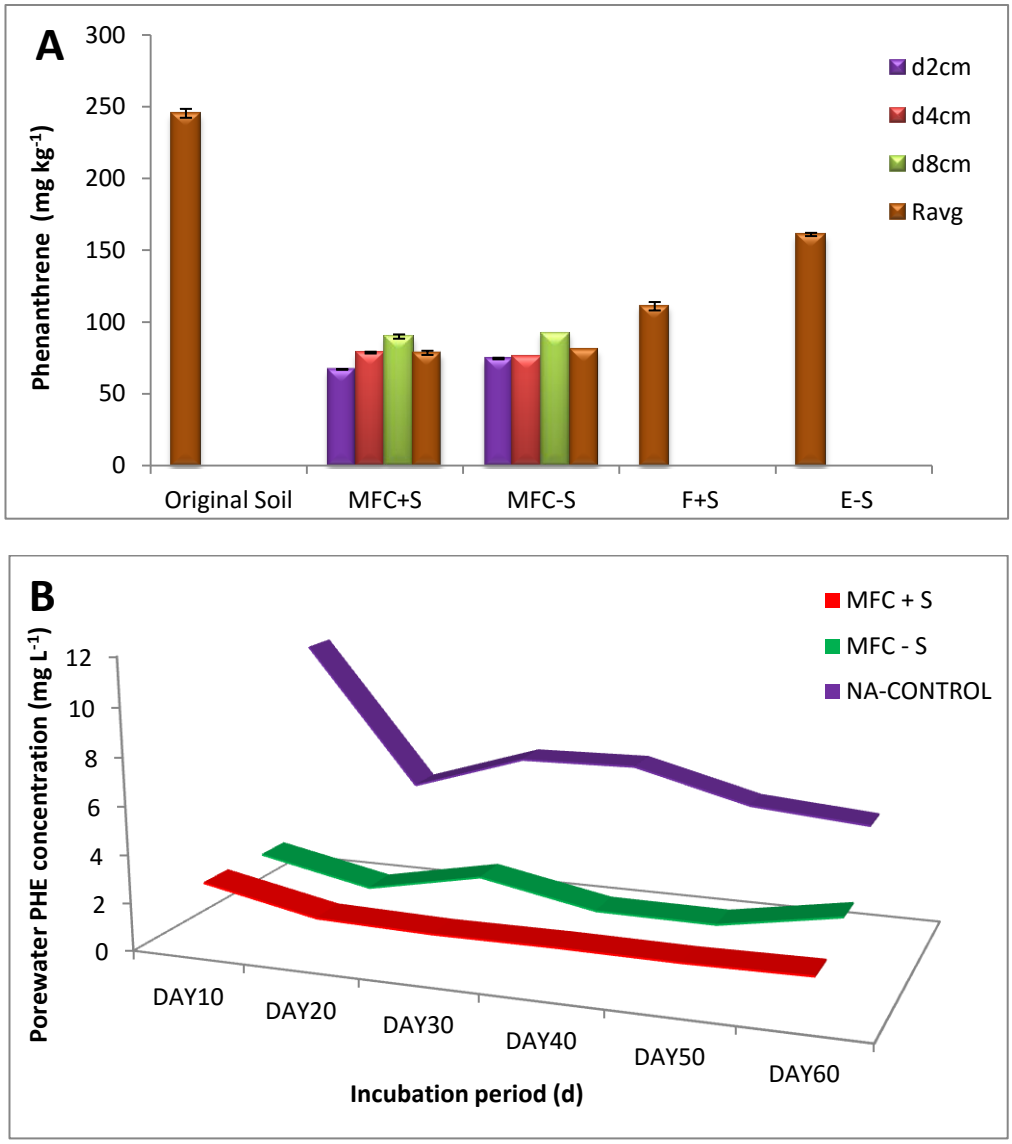


Figure 6.3: (A) Total phenanthrene removal at different distances from the anode at the end of testing period with MFC only (MFC-S) and MFC with surfactant (Tween 80, 250 mg L^{-1}) amendment (MFC+S). (B) Phenanthrene porewater concentration in the overlying water in the saturated soil MFC. Controls 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 removal of phenanthrene was similar to that of TCOD, as shown in Figure 6.4.

Figure 6.4 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 the MFC only (MFC-S), relatively compared to the baseline controls. 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 6.2). The slope of the ROI gradually becomes less negative with time, indicating a steady ROI's expansion with respect to time of reactor operation.

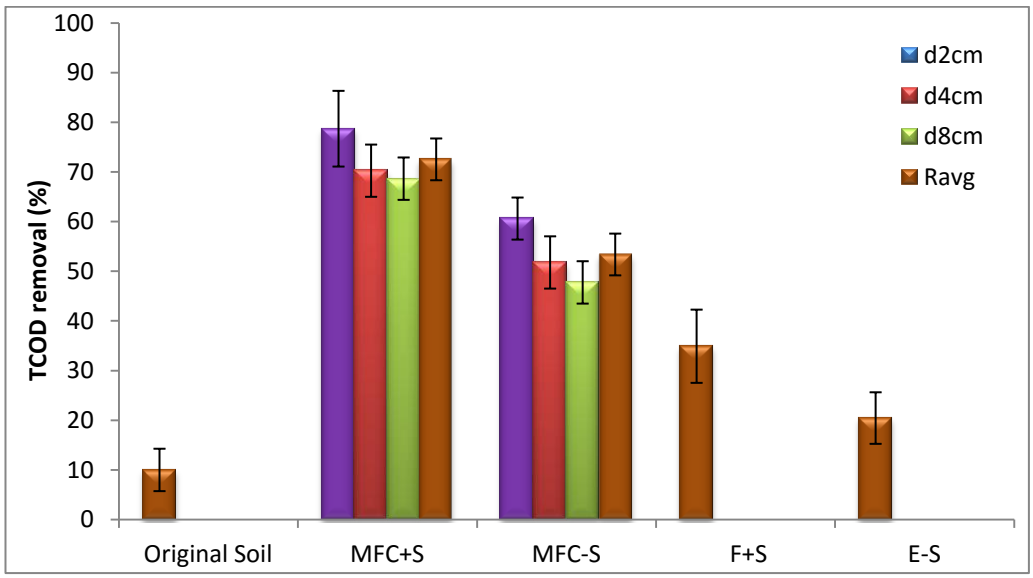


Figure 6.4: Percentage total COD (TCOD) removal at different distances from the anode for the active MFCs and control reactors at the end of 60 days of MFC operation at ambient temperature. Ravg is the average of removal efficiencies at different radial distance from the anode. Values are means of triplicate measurements ± SD. E-S and F+S mean controls without and with surfactant addition respectively.

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 60 day period of MFC operation (Figure 6.5). 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 transfer especially in soil systems accounted for possible reduction or oxidation of pollutants in open circuit MFCs reported previously by Huang et al (2011) and Nielsen et al (2010).

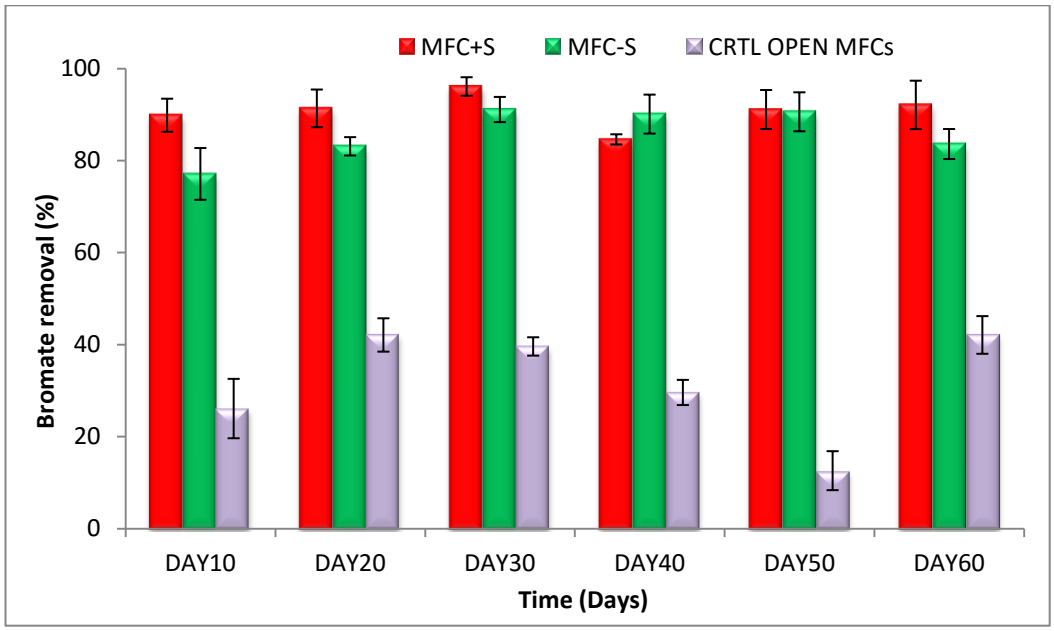


Figure 6.5: Bromate removal efficiency in the tubular MFC reactor during 60 days operation at incubation temperature between 25 and 30 ℃. The error bars ±SD were based on averages measured in triplicate.

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 substantially enhance phenanthrene biodegradation up to 293 % of that from the baseline reactor with the enhanced biodegradation from the MFC anodes extending even to the edge of the reactor (Figure 6.1). This finding demonstrates the capabilities of this MFC configuration in actual field applications to enhance biodegradation of petroleum contaminated soils coupled with bromate removal. This passive remedial technology is environmentally friendly and can shorten remediation time with potential cost savings.

6.2.2 Electricity 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 6.6. 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). After an initial lag phase, the electric current output profiles for the MFCs indicated its correlation with hydrocarbon biodegradation. 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 electricity generation results were similar to the previous reports on the responses of waterlogged-contaminated soils during electricity generation using MFCs (Huang et al., 2011; Wang et al., 2012b; Lu et al., 2014a). The gradual increase in current generation may be due to

microbial acclimation and increase in the activity of the electrochemically-active microbial population in the soil.

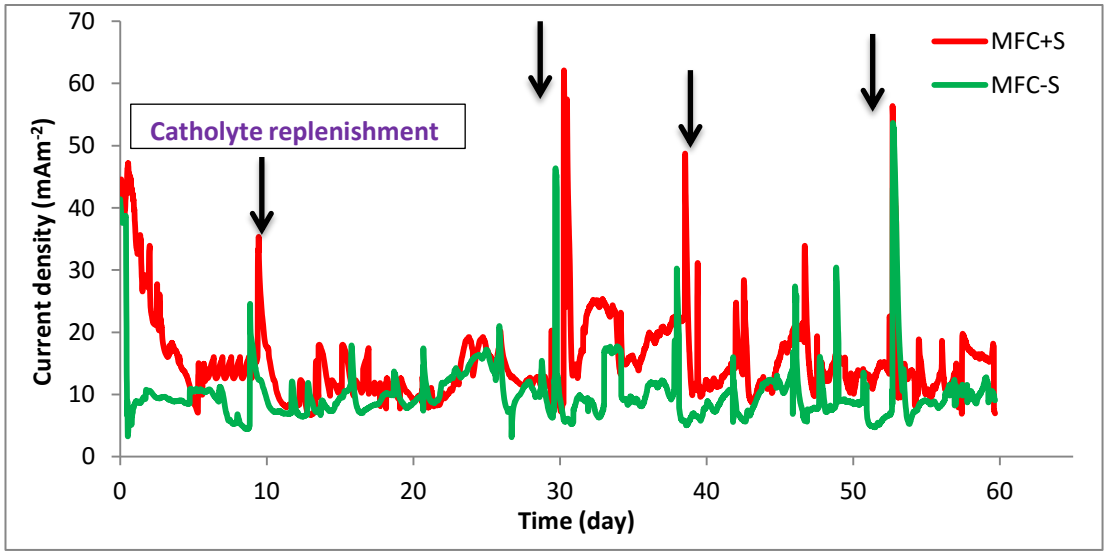


Figure 6.6: Current densities of MFC+S and MFC-S during the tubular MFC operations over 60 days incubation period (R_{ext} = 1000 Ω). Black arrows indicate the points for catholyte replenishment.

Current output during reactor operation was subject to fluctuation, probably due to the production of biotransformed intermediate products resulting from phenanthrene degradation and uneven mass transfer within the contaminated soil during the long operational periods (Lu et al., 2014b; Huang et al., 2011).

The CV (cyclic voltammograms) of the anode chamber of the MFC was analyzed during incubation at day 10 and 30 (Figure 6.7). 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 only as shown in Figure 6.7. This perhaps, might indicates 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 MFC, as shown in this study, could enhance phenanthrene removal and improve electrochemical performance of MFC. A similar observation was recently reported by Wu et al (2014) enhanced toluene degradation and power generation in MFCs amended with a surfactant, pycocyanin.

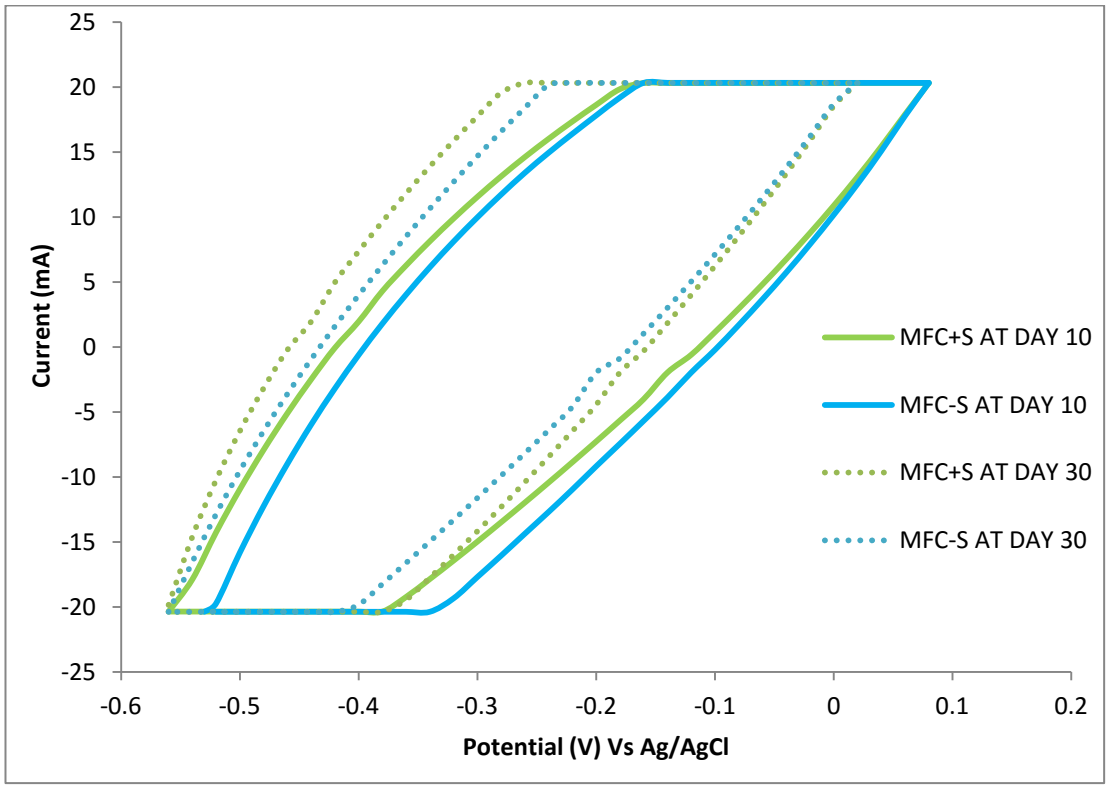


Figure 6.7: The CV curve for phenanthrene in MFC only (MFC-S) and MFC with surfactant (Tween 80, 250 mg L^{-1}) amendment (MFC+S) at day 10 (solid line) and day 30 (dashed line) at sweep rate of 10 mVs^{-1}.

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. The profile of the electric current in a MFC could potentially be used to remotely monitor the progress of biodegradation, eliminating field soil sampling frequency

and electricity generated during the biodegradation can be used as power for remote sensors.

6.2.3 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 6.8). The soil pH values for all the MFCs except the controls, decreased up to 0.23 pH units within the first 10 d at a radial distance of 2 cm from the anodes of each BES (relatively lower than those obtained at radial distances 4 and 8 cm, indicating a slight proton accumulation occurred close to 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 to 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., 2011; Allen et al., 2007). Notably, formation 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., 2007; Wang et al., 2012b). Soil microbial

activity declined as EC increased and this might greatly influence other important soil processes such as respiration, advection/adsorption, residue decomposition, nitrification and denitrification (Allen et al., 2007; Johnsen et al., 2005).

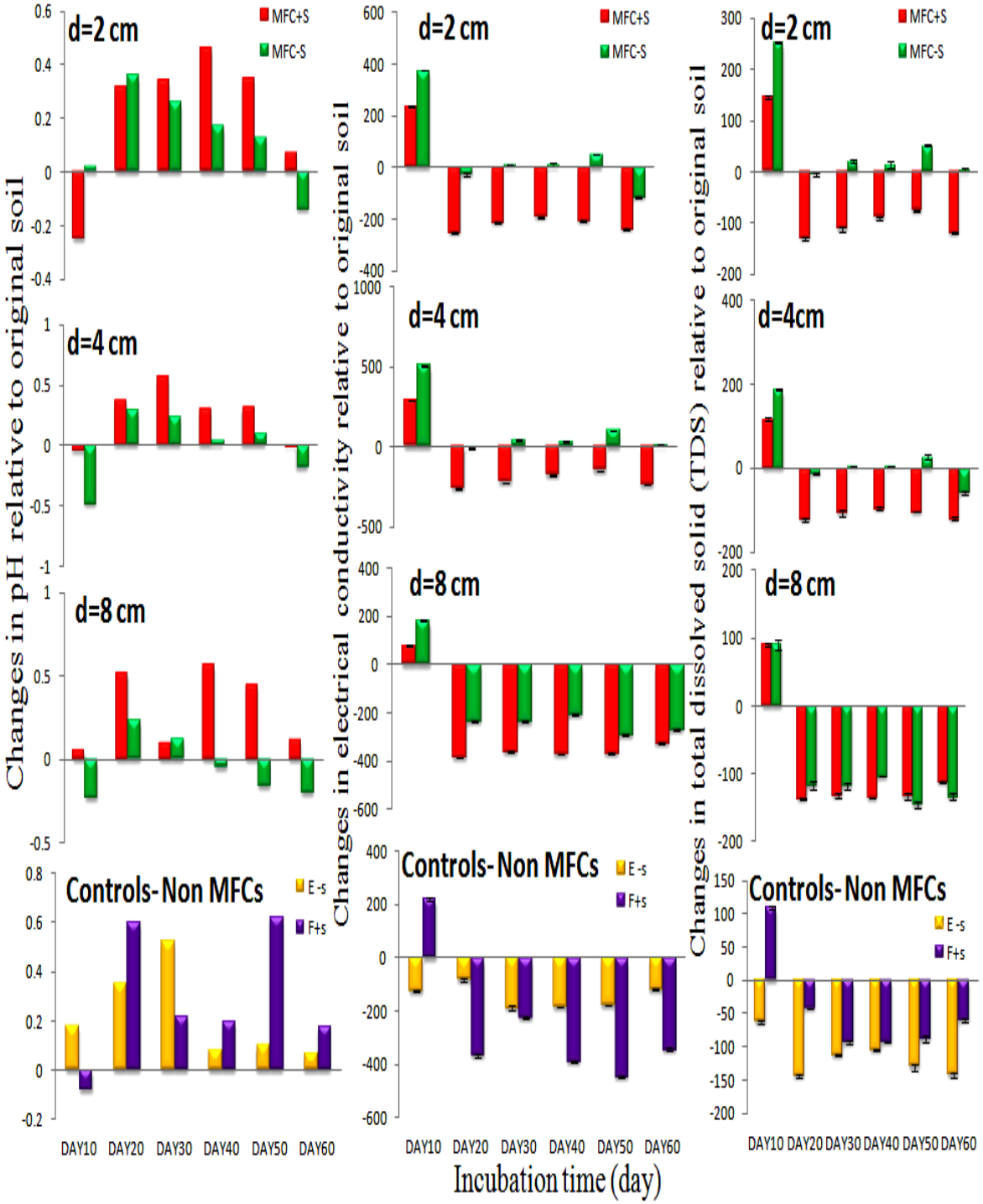


Figure 6.8: Changes in soil pH, TDS and electrical conductivity in MFC at different distance from anodes and control reactors over the tested period. The error bars ±SD were based on averages measured in triplicates. E-S and F+S mean controls without and with surfactant addition respectively.

Statistically, the parameters analyzed clearly 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 summarized in Table 6.1, phenanthrene removal is negatively correlated with TDS in the soil ($p < 0.05$) and 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, accompanied by phenanthrene degradation and bromate removal, provide a comprehensive profile on how MFC enhances hydrocarbon remediation coupled with bioelectricity generation and supports the restoration of the contaminated soil to its natural ecological status.

Table 6.1: 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).

6.2.4 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 poses no immediate danger to human health and ecosystems; the ultimate goal of any successful remediation process (Liu et al., 2010; Melo et al., 2013; Ayed et al., 2011). Microbial degradation of pollutants often results in incomplete mineralization and hence, 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 6.9.

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 only (MFC-S) respectively compared to baseline controls. From Figure 6.9, 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., 2014; Hamdi et al., 2007).

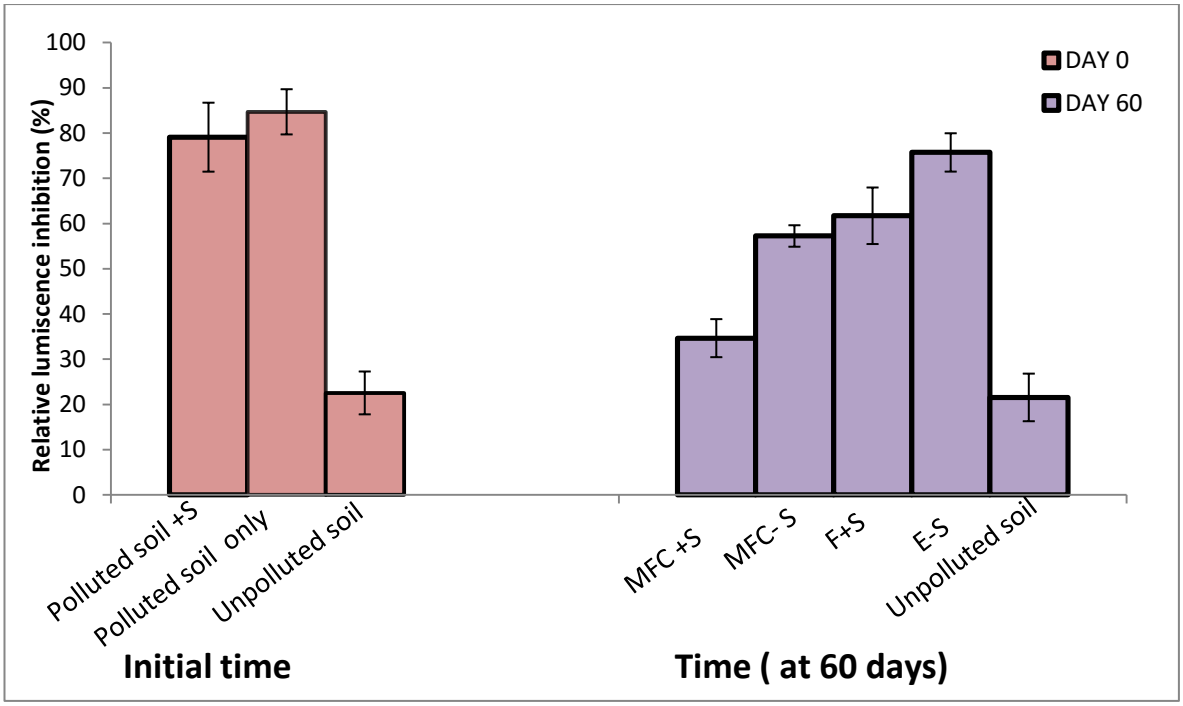


Figure 6.9: Toxicity levels in soil extractions of polluted soil with and without MFC treatment operated at 25-30℃ over 60 days incubation period. The error bars represent 5% of deviation of the mean value for triplicate measurements. MFC-S and MFC+S are MFC only and MFC with surfactant (Tween 80, 250 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 amendments towards a successful restoration/reclamation of the contaminated site (Hankard et al., 2004; Vogt et al., 2007; Sarkar et al., 2005).

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 conditions.

6.3 Concluding remarks

In this study the performance of a tubular MFC system 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. This study has demonstrated 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 such as monitoring wells or piezometers. Electricity generated can 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.

CHAPTER 7:

CONCLUSIONS

The overarching aim of the experiments presented in this thesis was to develop a laboratory-based bioelectrochemical system for the degradation of phenanthrene and benzene coupled with concomitant electricity generation that is effective, efficient, robust for different treatment conditions and applicable to liquid and particulate systems. In order to achieve this aim, four main studies were conducted with detailed discussions reported in chapters 3-6.

Chapter 3 focused on the degradation of benzene and phenanthrene in the anode of dual-chamber MFCs using a range of inoculum type (*Shewanella oneidensis* MR1 14063, *Pseudomonas aeruginosa* NCTC 10662, mixed cultures and their combinations thereof). For studies on phenanthrene degradation, the best overall performing inoculum was MCP, a mixed culture supplemented with *P. aeruginosa*. The culture gave a phenanthrene degradation rate of 4.93 mg $L^{-1}d^{-1}$, a maximum power density of 1.25 mWm^{-2} and a COD removal of 65.6 %. The bacterial consortium- AMC was the best performing inoculum for benzene degradation studies. The culture gave a benzene degradation rate, maximum power density and COD removal of 5.41 mg $L^{-1}h^{-1}$, 0.82 mWm^{-2} and 87.3 % respectively. This suggests that these selected best-performing strains may offer good prospects for bioaugmentation in MFCs for the treatment of petroleum hydrocarbons The EC_{50} values, based on *Vibrio fischeri* ecotoxicity testing, for post treatment samples was three-fold higher than the pre-treatment samples suggesting a lower cytotoxicity effect. The influence of carbon sources concentration on MFC performances, using two different inocula, was also investigated in this study. The results of this study revealed that reduction in substrate's concentration up to 100 mg L^{-1} has no significant impact on degradation performance. Hence, this suggests the use of lower

amount of carbon source in commercial applications; thereby reducing treatment costs.

The second part of this project (chapter 4) involved the studies on the robustness of MFCs at different treatment conditions with petroleum hydrocarbon-synthetic wastewater as fuel using hydrocarbons-acclimated mixed microbial consortia. Results of this study indicated the possibility of achieving over 95 % degradation efficiency in hydrocarbon mixtures, using an adapted anaerobic microbial consortium, in a repeatable and consistent fashion during fed-batch MFC operation. Efficient degradation of petroleum hydrocarbons was obtained at mesophilic (40℃) and moderately saline (1 % w/v NaCl) conditions. MFC performance in terms of electricity generation was enhanced when exogenous redox mediator such as riboflavin were added. Optimal power density of 10 mWm^{-2} was obtained at an applied external resistance of 100 kΩ while maintaining a good degradation performance. Electrochemical performance of the MFC was enchanced significantly when potassium bromate was used as catholyte without deterioration in degradation performance. This suggests the use of bromate in MFCs as catholytes or electron acceptors in lieu of expensive and non-renewable metal catalyst is possible. Triton X100 has lower toxic effect relative to Tween 80. The addition of surfactant within the range tested increased the apparent aqueous solubilty of phenanthrene but do not enhance phenanthrene removal efficiency. Studies on the interactive effect among selected operating factors (namely; salinity, redox mediator and external resistance) using RSM revealed that the interactive effects exist among the chosen independent factors with external resistance having a significant impact on MFC performance, especially in terms of power outputs. Results demonstrated that RSM as a useful and effective tool can be employed in evaluating and optimising MFC performance,

hence providing guidance for future deployment of MFC systems in field applications.

BES systems need to be designed uniquely and tested for their performance before possible deployment for either *in situ* or *ex situ* applications. Two Tubular MFC designs for both *in situ* and *ex situ* applications in aqueous systems were investigated and discussed in Chapter 5. Simultaneous removal of petroleum hydrocarbons and bromate with concomitant biogenic electricity generation was obtained optimally at HRT 10 d. However, the tubular MFC was successfully operated at high (100 mg L^{-1} phenanthrene, 2000 mg L^{-1} benzene at HRT 30) and low (phenanthrene and benzene, 50 µg L^{-1} for each) substrate levels suggesting its effectiveness and robustness at hostile operating conditions in anoxic environments, especially deep groundwater reservoir. In MFC designed for future *ex situ* applications, 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. MFC reactor had exhibited the ability to resist organic shock loading or high concentrations (100 mg L^{-1} phenanthrene and 2000 mg L^{-1} benzene) and maintain a stable performance under high substrate levels. Results of this study 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) conditions.

In the work described in Chapter 6, the performance of a lab-scale MFC (a tubular design) for enhanced bioremediation of phenanthrene-contaminated soil was investigated. This study has demonstrated the simultaneous removal of phenanthrene and bromate coupled with concomitant bioelectricity generation using MFC systems with a projected maximum ROI up to 40 cm. Electricity generated during pollutant degradation in MFCs could be used to power wireless sensors for remote site

monitoring and as an indicator for real-time contaminant degradation profiling. MFC technology could be deployed directly as a prototype-MFC design in field applications or integrated with existing infrastructure such as monitoring wells or piezometers for *in situ* soil bioremediation, especially in subsurface soil environments with depth ranging from 2 to 10 m.

In final conclusion, MFC systems were designed and tested under different operating/environmental conditions for the treatment of petroleum hydrocarbons in contaminated soil and water systems. Knowledge of the robustness of these designed MFCs to different operating conditions is very important in assessing the technical feasibility of these systems if they are to be applied in the field for bioremediation and compete with other conventional technologies. The outcome of the studies presented in this thesis implies possible application of MFC technology in the effective treatment of petroleum hydrocarbons contaminated groundwater or industrial effluents and soil systems (mostly in subsurface environments) robustly with concomitant energy recovery. MFC technology could potentially be utilised as an independent system in lieu of other bioremediation technologies (e.g. pump and treat or permeable reactive barriers) or integrated with existing infrastructures such as monitoring wells in the treatment of petroleum hydrocarbons in contaminated subsurface environments or industrial effluents.

CHAPTER 8:
FUTURE WORK

MFCs still have a long way to go to reach application at large scale. Based on the results obtained in this project, there are several potential areas that could be investigated in the future for the development of bioelectrochemical systems in the treatment of petroleum hydrocarbons contaminated soil or water.

8.1 Studies on the MFC performance in different soil types.

This study has demonstrated the treatment of phenanthrene-contaminated soil using bioelectrochemical systems. In this study, a waterlogged sandy-loam soil was used. However, in natural environments, soil compositions or types vary spatially depending on the climatic conditions of the area under investigation (Juana et al., 1998). Soil physico-chemical characteristics such as soil pH, moisture content, soil structures e.t.c varies widely depending on climatic conditions (that is geographically based) and seasonal variations which in turn could have profound impact on soil MFC performance and their subsequent field applications. Only few studies on soil MFCs have been reported in the literature (Huang et al., 2011; Morris and Jin, 2012; Lu et al., 2014b; Wang et al., 2012b). Therefore, for more robust implementation of MFCs systems in various field applications regardless of the soil conditions, further studies should be conducted on the effect of soil types, temporal and seasonal variation on soil MFC performance over a long period of operation.

8.2 Scale-up operation for MFC systems designed for aqueous system and field trials

For future commercialisation of MFC systems, there is need for further studies on MFC performance in large scale systems using real contaminated wastewater and/or groundwater. The use of real wastewater or groundwater is required in order to better evaluate MFC performance and optimise operating conditions using statistical method such as response surface analysis.

8.3 Enhancing electricity generation in MFC by improving MFC configuration and appropriate selection of good materials used in MFC systems.

Bioelectricity generation in MFC systems is an added economic value that makes BES technology promising and attractive compared to other conventional technologies as power generation is associated with pollutant removal either at the anode, cathode or both. However, in this study, the maximum power generated is still low and needs to be further improved. MFC design and the nature of materials used influence the electrochemical performance of BES systems immensely. Therefore, future studies could focus on improving MFC architecture design and materials in order to increase efficiency and power production while maintaining the extent of petroleum hydrocarbon removal. This new approach will greatly improve groundwater and soil remediation efficiency and may ultimately reduce costs (in terms of energy recovery).

8.4 Improving cathodic performance in MFC systems.

In this study, the use of bromate as an alternative terminal electron acceptor in lieu of Pt/O_2 cathode has been demonstrated for the first time and subsequently been found to be effective in enhancing power generation and pollutant removal efficiency in MFC systems. Bromate is an oxidising agent with higher redox potential than oxygen and it has been applied to both aqueous and soil MFCs in some studies reported in this thesis. Further studies on bromate as a replacement for Pt-cathode in MFCs is required in order to optimise cathodic reduction efficiency and the overall MFC performance. Studies such as the effect of increase in bromate concentration, pH on MFC performance and the effect of bromate as catholyte over a long period of MFC operation on microbial community structure at the anode need to be

investigated. Also, due to high cost of platinum which is used in making chemically-catalyzed cathode, possible replacement with the use of biocathodes would alleviate significantly the high cost of BES reactor design in large-scale field applications. Therefore, the investigation of algae as possible biocathodes is recommended as algae can produce *in situ* oxygen and other reactive oxygen species required for improving cathodic reduction reaction. Examples of algae that can be used in this future study include green and red algae. Cyanobacteria (blue-green algae) are also group of prokaryotic microorganisms which are very close to algae, that could can be investigated in future MFC studies.

CHAPTER 9:

APPENDIXES

APPENDIX 1

Table 9.1: 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 (0-2 µm)	9
% Silt (2-63 µm)	28
% Sand (63 µm - 2 mm)	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	15
Background phenanthrene (mg/kg DS)	1.950
Water soluble anions (mg/kg DS):	
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/kg DS):	
K	440
Ca	3124
Mg	92
Na	9.7
Al	0.61
Fe	0.93
Mn	0.06
Exchangeable cations (cmol(+)/kg DS):	
K	1.124
Ca	15.590
Mg	0.754
Na	0.042
Al	0.007
Fe	0.005
Mn	0.000

DS: Dry soil

APPENDIX 2

The standard curve was prepared using a bromate standard over a concentration of 0-1000 mg L^{-1} using UV-visible spectrophotometer at 590 nm.

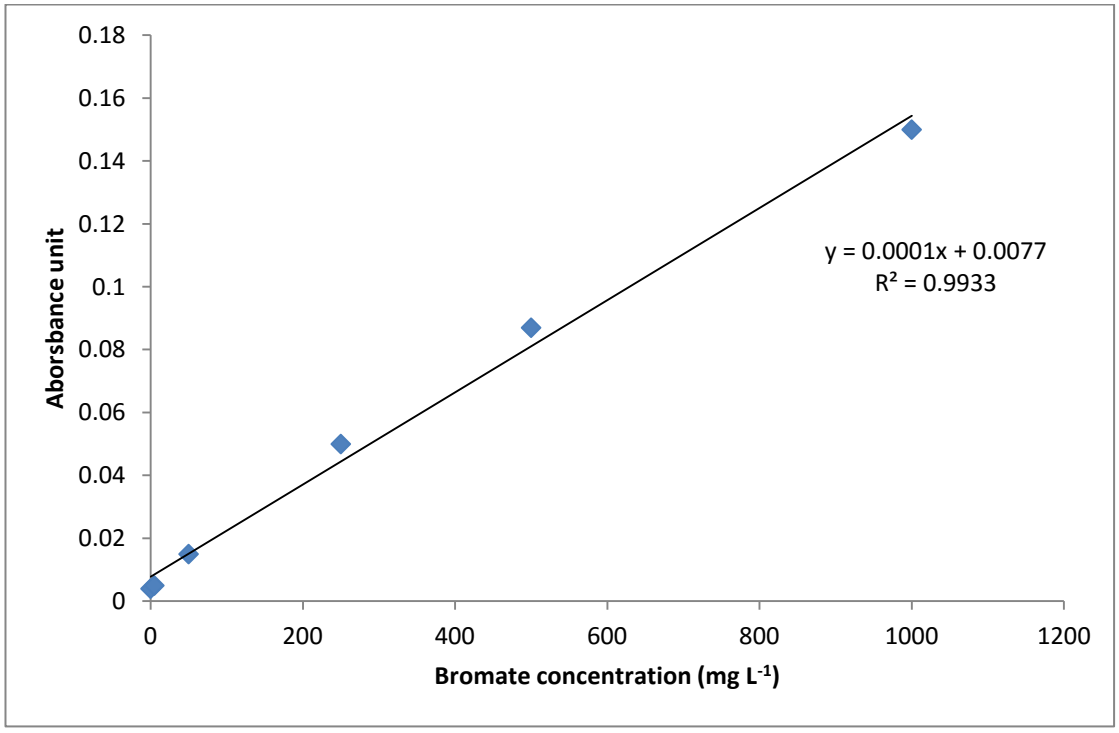


Figure 9.1: Calibration plot for bromate standard concentrations

APPENDIX 3

The analysis of variance (ANOVA) for response surface quadratic polynomial model for power density.

Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)	
Model	844.32	9	93.81	29.88	< 0.0001	significant
A-Salinity	10.52	1	10.52	3.35	0.0971	
B-Ext. resist.	656.91	1	656.91	209.22	< 0.0001	
C-Redox mediator	0.015	1	0.015	4.687E-003	0.9468	
AB	9.32	1	9.32	2.97	0.0157	
AC	0.40	1	0.40	0.13	0.7298	
BC	0.67	1	0.67	0.21	0.0551	
A^2	4.08	1	4.08	1.30	0.2808	
B^2	0.33	1	0.33	0.11	0.7509	
C^2	23.66	1	23.66	7.54	0.0207	
Residual	31.40	10	3.14			
Lack of Fit	31.40	5	6.28			

ANOVA for Response Surface Quadratic model:TPH

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	311.09	9	34.57	2.26	0.1101	not significant
A-Salinity	*12.84*	*1*	*12.84*	*0.84*	*0.3812*	
B-Ext. resist.	*16.18*	*1*	*16.18*	*1.06*	*0.3280*	
C-Redox mediator	*1.34*	*1*	*1.34*	*0.088*	*0.7733*	
AB	*2.87*	*1*	*2.87*	*0.19*	*0.6743*	
AC	*0.96*	*1*	*0.96*	*0.063*	*0.8074*	
BC	*0.33*	*1*	*0.33*	*0.022*	*0.8858*	
A^2	*32.46*	*1*	*32.46*	*2.12*	*0.1759*	
B^2	*2.20*	*1*	*2.20*	*0.14*	*0.7124*	
C^2	*24.68*	*1*	*24.68*	*1.61*	*0.2328*	
Residual	152.99	10	15.30			
Lack of Fit	*152.99*	*5*	*30.60*			
Pure Error	*0.000*	*5*	*0.000*			
Cor Total	464.07	19				

Quadratic model:TPH

Total TPH (%)

$$= 58.35 - 1.16A + 1.27B - 0.37C - 0.55AB + 0.35AC + 0.19BC + 3.44A^2 + 28.18B^2 + 3.00C^2 \quad \text{..... (27)}$$

where A is salinity (NaCl concentration), B is external resistance and C is concentration of the redox mediator (riboflavin

ANOVA for Response Surface Linear model: COD removal

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1946.97	3	648.99	1.23	0.3315	not significant
A-Salinity	*604.35*	*1*	*604.35*	*1.14*	*0.3005*	
B-Ext. resist.	*0.50*	*1*	*0.50*	*9.451E-004*	*0.9759*	
C-Redox mediator	*1342.12*	*1*	*1342.12*	*2.54*	*0.1304*	
Residual	8445.77	16	527.86			
Lack of Fit	*8445.77*	*11*	*767.80*			
Pure Error	*0.000*	*5*	*0.000*			
Cor Total	10392.74	19				

Linear model: COD removal

$$\textbf{COD removal (\%)} = 59.41 - 7.77A + 0.18B - 11.59C \quad \text{.....(28)}$$

where A is salinity (NaCl concentration), B is external resistance and C is concentration of the redox mediator (riboflavin).

Appendix 4

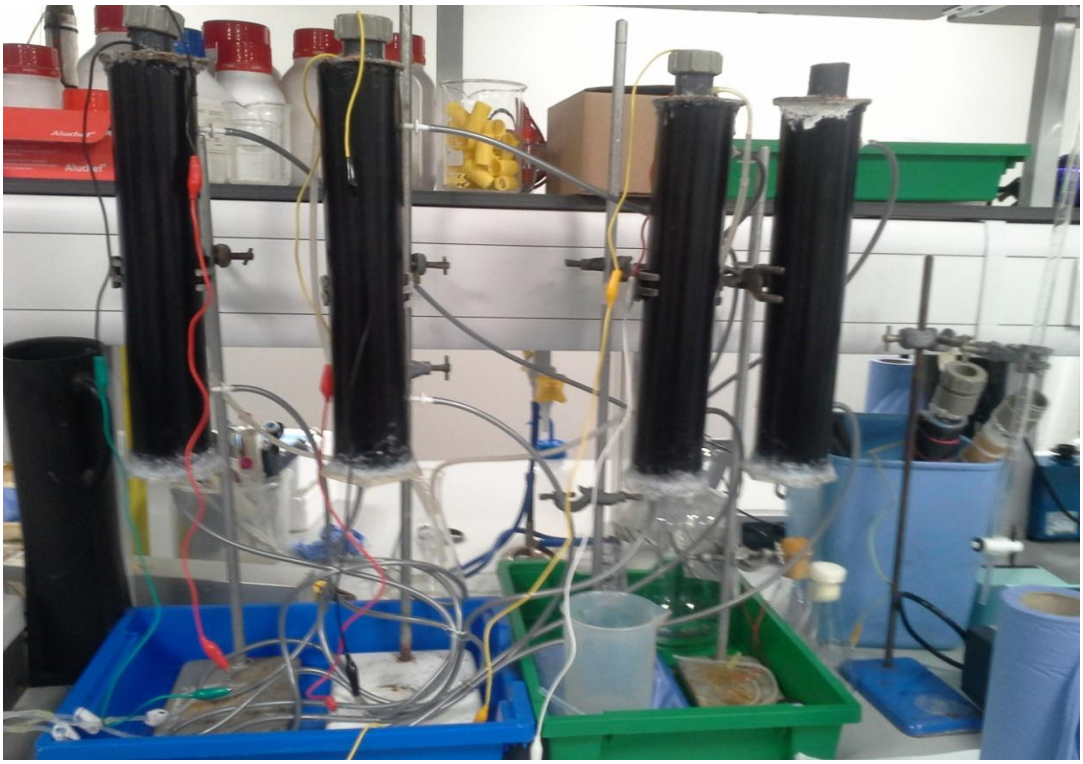


Figure 9.2: The experimental set-up during the start-up stage of the continuous run of the tubular MFC system for the treatment of synthetic wastewater containing petroleum hydrocarbons.

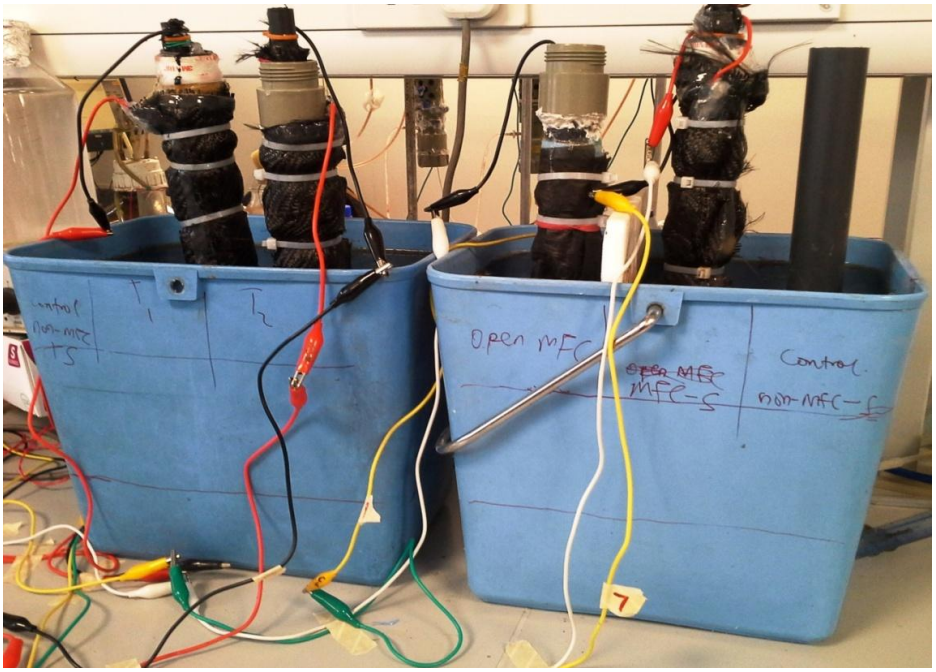


Figure 9.3: (A) The soil MFC system during start-up for treating a model PAH-contaminated soil

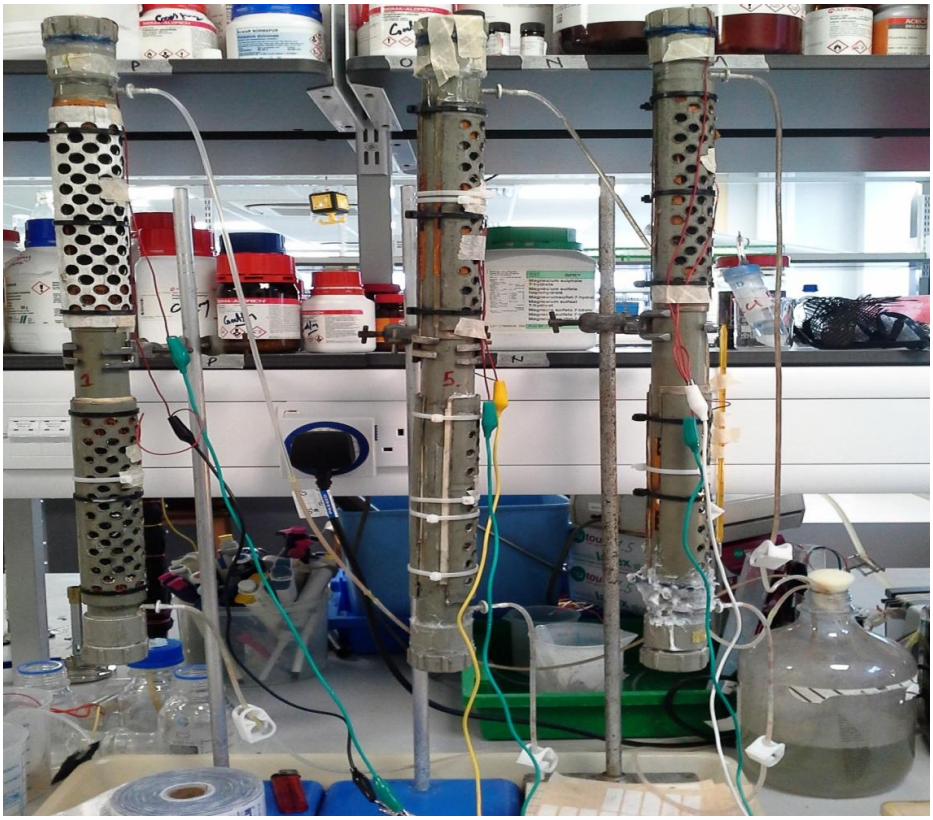


Figure 9.4: The MFC system (during start-up of the continuous run) used for the treatment of petroleum hydrocarbon containing synthetic wastewater.

CHAPTER 10:

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