Strategies for enhancing bioelectrochemical remediation of petroleum hydrocarbons

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STRATEGIES FOR ENHANCING BIOELECTROCHEMICAL REMEDIATION OF PETROLEUM HYDROCARBONS

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Abstract

Globally, Petroleum Hydrocarbons (PH) contamination in the environment is widespread due to accidental spillages, leakages and indiscriminate disposal. PH pollutants are continuously a concern because of their high toxicity to humans and the environment. While there have been efforts to remediate these pollutants from the environment, the conventional approaches are very slow, expensive and energy-intensive among other challenges. These necessitate the quest for remediation approaches that are cost effective, environmentally friendly and sustainable. Recently, there have been efforts to develop new or enhance existing biological approaches that are considered largely as better alternatives to other remediation methods. Bioelectrochemical System (BES), an emerging biotechnology approach with a wide range of applications, has been adjudged as one of the cost-effective and environmentally friendly methods that has the potential to enhance faster degradation of petroleum hydrocarbon pollutants. Despite its potential, MFC is faced with several limitations. To this end, BES research aimed at enhancing the degradation of petroleum hydrocarbon pollutants was carried out in this study.

Samples from PH polluted matrices (groundwater, sediment and soil) in the Niger-Delta region of Nigeria were collected. A metagenomic analysis of these samples showed that the phylum Proteobacteria was dominant in soil and groundwater while Campilobacterota was dominant in the sediment. Using two culture-based approaches and screening pressures, PH degraders that are facultative anaerobes with good electrochemical activity and but not mandatory that could produce biosurfactant were isolated. Each isolate was identified using 16s rRNA region to exclude any potential pathogen. 5 isolates across the matrices produced biosurfactant with emulsification Index ranging from 30%-99%. When compared to a negative control and positive control (*Shewanella oneidensis*), several isolates had good electrochemical activity (peak currents) that were greater than that of *S. oneidensis* by about 24.2-194.5%. After the screening, 14, 11 and 6 isolates were selected from soil, sediment and groundwater respectively Microbial Fuel Cell (MFC) experiments.

MFC experiments augmented with microcosms were compared with MFC augmented with single pure strain from each matrix. The result of the MFC variables (i.e. voltage over time and power density) for microcosm experiments showed a better performance compared to that of the pure strains. Hence, the microcosm integration was selected for MFC experiments. Subsequently, bioreactors containing waterlogged soil polluted with 200000 µg of benzene and 100000 µg of phenanthrene in three different experimental design were set ups. The first that contained an MFC, microcosm and 5% biochar and the second that contained MFC and microcosm had the best performance that degraded 200000 µg and 100000 µg of benzene phenanthrene completely within six days while the negative control degraded about 6300 µg and 34.8 µg of benzene and phenanthrene respectively. The overall results from the experiments suggest that augmentation of the right microcosm with BES could be the game changer in the remediation of petroleum hydrocarbon.

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Conference presentations

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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: 31st January, 2024.

Belinda Yoba'a Yayork

List of Abbreviations

ATSDR Agency for Toxic Substances & Disease Registry
BES Bioelectrochemical system
BLAST Basic Local Alignment Search Tool
BP British Petroleum
BTEX Benzene, Toluene, Ethyl benzene and Xylene
CV Cyclic voltammetry
DNA Deoxyribonucleic acid
EAB Electro Active Bacteria
EET Extracellular electron transfer
EIA Energy Information Administration
EU European Union
HPLC High Performance Liquid Chromatography
HYPREP Hydrocarbon Pollution Remediation Project
ITOPF International Tanker Owners Pollution Federation
MECs Microbial electrolysis cells
MFCs Microbial fuel cells
MHA Mueller Hinton Agar
NAPLs Non-Aqueous Phase Liquids
NCBI National Centre for Biotechnology Information
OPEC Organization of the Petroleum Exporting Countries
OTUs Operational Taxonomy Units
PAHs Polycyclic aromatic hydrocarbons
PCR Polymerase chain reaction
PEM Proton exchange membrane
RNA Ribonucleic acid
ROI Radius of influence
rpm Revolutions per minute
rRNA Ribosomal RNA
TPH Total Petroleum Hydrocarbon
UNDP United Nations Development Programme
UNEP United Nation Environment Programme
USEPA US Environmental Protection Agency
WHO World Health Organisation

Chapter 1: Literature review

Introduction

The petroleum industry plays a key role in meeting the global energy need for both industries and everyday life. In 2018, the growth rate of the global primary energy consumption was at 2.9% per annum. Among the six primary energy types consumed, petroleum products ranked the highest with 4662.1Mtoe while renewable energy was least with 561.3Mtoe (BP, 2019). Despite the global efforts towards transitioning from a carbon-intensive fossil fuels based-system to a low carbon (renewable energy) based-system (World Energy Council, 2019) due to fossils fuels' adverse effects on both human health and the environment, it has been projected that fossil fuels will remain a major source of global primary energy in which daily demand for crude oil will be 120 million barrels a day by 2050i OPEC (2024). With the current global estimate of close to a billion people who do not have access to electricity, three billion without modern fuels for cooking (OPEC, 2019), and the continual increase in the world population, crude oil can be expected to remain a primary energy source even beyond 2050. Unfortunately, during crude oil exploration, production, storage and transportation, crude oil-based products are released into the environment through accidents, spills and leaks (Das and Chandran, 2010). Between 1970 to 2018, about 5.8 million tonnes of oil has been spilled because of tanker incidents globally (ITOPF, 2019) and between 1.7 to 8.8 million tonnes of oil is said to be released into the global aquatic environment annually (Dadrasnia and Agamuthu, 2013). Seepage is one of the culprits of environmental pollution by crude oil and it is estimated to contribute 600,000 tonnes per annum with an uncertainty range of 200,000 metric tonnes (Kvenvolden and Cooper, 2003). The 2010 Deepwater Horizon oil spill in the Gulf of Mexico and the recurrent PH spills in the Niger Delta region of Nigeria are good examples of spillages from anthropogenic activities that have resulted in devastating impacts on the environment and human health. In the Niger delta, between 9 and 13 million barrels

of oil seepage were reported at over 2000 locations since the commencement of oil exploration and exploitation. These spills have resulted in the contamination of groundwater by benzene and polycyclic aromatic hydrocarbons at high concentration levels that are 800 and 500 times more than the WHO standards respectively.

The major constituents of crude oil are aromatic hydrocarbons and aliphatic hydrocarbons. The aliphatic hydrocarbons mainly include alkanes, cycloalkanes and alkenes while the aromatic hydrocarbons are Polycyclic Aromatic Hydrocarbons (PAHs) and Benzene, Toluene, Ethylbenzene, Xylenes (BTEX). The minor constituents are nitrogen, sulphur, oxygen and trace quantities of metals (Gary et al., 2007; Speight, 2014). Commonly known products derived from crude oil are diesel, paraffin wax, lubricating oil asphalt and gasoline (Altgelt, 2016, as cited in Wang et al., 2020). Environmental pollution by petroleum hydrocarbons (Figure 1.1) is considered a priority due to its negative impacts on human health and the ecosystem (UNEP, 2011; Sajna et al., 2015; Varjani, 2017). The long exposure of people to a group of crude oil compounds known as BTEX (benzene, toluene, ethylbenzene, xylene) is said to have the potential of causing cardiovascular and respiratory illnesses, affect the development and function of the immune system, reproduction system and metabolism (WHO, 2000; El-Hashemy and Ali, 2018; Pouresmaeili et al, 2018; Latif et al, 2019).

(a) (b)

Figure 1.1 (a)l pollution in Ogoniland, Nigeria; (a) aerial view of an oil spill; (b) oil-contaminated soil (UNEP, 2011)

Other impacts include neurological impairment, increased risks of cancer and haematological effects such as acute myelogenous leukaemia and aplastic anaemia (WHO, 2000; ATSDR, 2004; Marc et al., 2016). Furthermore, short-time exposure to high concentrations of BTEX may trigger circulatory mortality (Ran et al., 2018). Another group of crude oil compound known to be a threat to human health is the Polycyclic Aromatic Hydrocarbons (PAHs). This group of compounds is also a priority due to their genotoxic, mutagenic and/or carcinogenic characteristic (Ghosal et al., 2016). Furthermore, crude oil spills can lead to irreversible habitat loss and threat to the survival of organisms living within the habitats. This pollution also threatens food security as root crops such as cassava that grow in polluted areas are not considered safe for consumption (UNEP, 2011), affect the germination of seeds, growth of crops and also reduce crops' yield (Chukwuka et al., 2018). PH pollutants are also prioritized because of their recalcitrant nature (ATSDR, 2011). Prevention measures are often employed to ensure that these contaminants do not get into the environment but when they do,

different strategies are employed to remediate contaminated sites. The approaches include physical, biological and chemical methods (Lim et al. 2016).

1.1 Methods for remediating petroleum hydrocarbon contaminated sites

1.1.1 Chemical methods

This involves the use of chemical reagents to remediate contaminated environments. Chemical dispersants have been used to emulsify petroleum hydrocarbons in contaminated water and soil (USEPA, 2017). When the dispersants get in contact with the pollutant, the large layer of the polluted oil is fragmented into smaller sizes of droplets thereby, increasing the surface area. The increased surface area plays an important role in enhancing the rates of the degradation of the pollutant (Brakstad et al., 2015). Dispersants have been used in several remediation processes with one of the largest quantities used estimated at of 6.97 million Litres Corexit for the Deepwater Horizon oil spill remediation. More quantity of the dispersant was applied at the depth rather than the surface (National Commission, 2011). The disadvantage of this method is its non-effectiveness at dispersing hydrocarbons with heavier molecular weight and its toxicity to the environment (USEPA, 2017). Furthermore, this method does not remove the pollutants but only disperses them for degradation by natural processes or other enhanced degradation methods. Another chemical method is chemical oxidation. This involves the use of chemicals e.g., hydrogen peroxide to oxidize the contaminants at the site of pollution (in situ). This method has enjoyed wide usage for both contaminated soil and groundwater (Usman et al., 2012). Although the reaction is fast, this is often short-lived and requires several injections of hydrogen peroxide that may lead to the production of by-products that can inhibit further degradation of the contaminant (Rico-Martínez et al., 2013). Another demerit of this method is that oxidized products such as

quinones, catechols and dihydrodiols can cause secondary pollution that can be more toxic than the primary pollutant thereby resulting to lower degradation rates because of inhibition of microbial activities (Wen-xiang et al., 2007). Furthermore, because this approach relies only on the contact between the contaminant and the injected oxidants, it is less effective in low permeability matrices such as clay-rich soils that require invasive methods like soil mixing (Usman et al., 2012) and the handling and shipping of hazardous chemicals for remediation can be difficult and expensive (Siegrist et al., 2011). Another chemical method is chemical precipitation. This involves the use of coagulating and/or flocculating agents such as lime, sulphides, calcite, caustic soda, spill-sorb and soda ash. These agents are used for the removal of colloidal and suspended pollutants but are not effective for remediating dissolved components (Juana et al., 1998; Frick et al., 1999). Other chemical methods include dissolved air precipitation and electrochemical processes among others. These methods are often technologically complex, expensive and with little or no public acceptance (Lundstedt, 2003).

1.1.2 Physical methods

Physical methods of remediation are also referred to as mechanical methods. They can either be in form of barriers that are used to prevent the oil spill from spreading (Ndimele et al., 2018) or in a form that alters the matrix of the pollution (Wang et al., 2020a). The following methods are:

1.1.2.1 Booms

These are techniques used to contain oil spills by enclosing floating oil and preventing it from spreading. Furthermore, they are employed to divert oil from environments with rich biological resources to environments with the least biological resource to reduce the negative environmental impacts of the spill. Booms are also used to concentrate the spilled oil and maintain a substantial thickness in an enclosed area for further remediation by other

techniques e.g. skimmers. This method contributes to oil recovery from the oil spilled environment and is usually more effective when used immediately after a spill. Booms are usually designed as either curtain or fence booms and are mostly arranged in a U configuration to enable containment (Michel and Fingas, 2015).

1.1.2.2 Skimmers

Skimmers are used to transfer the spilled oil floating on water from booms into storage tanks. This process does not alter the chemical or physical properties of the oil. Hence, it is used for oil recovery. They are categorized into suction, weir and oleophilic skimmers. Although skimmers have been found to be effective in calm waters, floating debris can clog them. Their effectiveness depends on the thickness and type of oil, weather and relies on surface tension, specific gravity and a moving medium for floating oil removal (Ndimele et al., 2018).

1.1.2.3 Adsorbent Materials

Adsorbent materials are mostly used to mop up the remaining oil after skimmers have been used. They help in converting the liquid oil to semisolids. These adsorbents can be natural (organic and inorganic) or synthetic materials. Natural organic sorbents include vegetable fibres, peat, hay, feathers, sawdust, milkweed, straw, etc. These adsorbents are capable of mopping up 3 to 15 times their weight in oil and are readily available and less expensive. The major challenge is the recovery of the adsorbents for proper disposal after the adsorption process has been completed (Ndimele et al. 2018). Natural inorganic adsorbents include clay, vermiculite, perlite, sand, glass wool or volcanic ash. They can absorb 4 to 20 times their weight in oil. They are not expensive and are readily available. The synthetic sorbents are man-made plastic-like materials such as polyethylene polyurethane and polypropylene. Most of these synthetic adsorbents can absorb 70 times their weight in oil (USEPA, 2017). Physical methods employed for the remediation of oil spills on soil include soil excavation, capping

and thermal-assisted extraction. These methods change the pollution matrix without necessarily destroying the contaminants. Another approach is soil vapour extraction that is carried out at the site of the pollution (in situ). This method has been reported to be less attractive due to its energy demand and the carbon footprints associated with it (Ávila-Chávez and Trejo 2010).

1.1.3 Thermal method

This method uses specialised booms and igniters that are fireproof to burn spilled oil floating on water. The method is most successful with refined oil products that are burnt readily, and the method is usually carried out immediately after a spill incident occurs. Before this method is carried out, it is important that floating vessels are not close to the remediating site. The major risk associated with this method is secondary fires that can cause devastating destruction to vegetations, aquatic lives, etc. Prolonged burning and emission of gases can increase risks to public health and the environment. This method is usually affected by water temperature, wave amplitude, speed, wind direction, oil type, slick thickness and the amount of weathering and emulsification that have occurred (Tewari and Sirvaiya, 2015).

1.1.4 Biological methods

These methods utilise biological agents (e.g. plants, microorganisms, enzymes etc) to remediate the environment contaminated by petroleum hydrocarbons. Biological techniques such as phytoremediation and bioremediation are used for cleaning up oil spills from polluted environments (Ndimele et al., 2018).

1.1.4.1 Phytoremediation

Phytoremediation is a process in which plants in association with microorganisms are used to decontaminate polluted environment (soil, water and air) by accumulating, degrading and

stabilising the pollutant Rajkumar et al., 2012; Cameselle et al., 2019). This technology has also been considered as a viable method for remediating polluted environment because its ease of implementation, cheaper compared to other remediating methods and public acceptability (Mandal et al., 2014; Liu et al., 2018). However, there are challenges associated with this that include: it requires lots of time, slow growing plants, and phytotoxicity when exposed to certain toxic compounds (Ashfaque et al. ,2016)

1.1.4.2 Bioremediation

This method involves the use of microbial biological mechanisms to clean up or detoxify polluted environments (Chang et al., 2002; Milic et al., 2009; Hernández et al., 2011). The strategies for bioremediation are grouped into natural attenuation (intrinsic bioremediation) and engineered bioremediation. Natural attenuation occurs when indigenous microorganisms naturally degrade recalcitrant pollutants. The demerit of the process is that degradation is slow. Hence, may take many years (Kuiper et al., 2004). Engineered bioremediation on the other hand, is the integration of several strategies such as bioaugmentation, biostimulation or combined strategies that greatly enhance biodegradation. The faster degradation rate of engineered bioremediation is its greatest advantage over natural attenuation (Talley and Sleeper, 1997; Taccari et al., 2012). Bioremediation plays a vital role in the decontamination of soils, groundwater and sediments that are polluted with hazardous organic compounds. However, there is no single bioremediation method that can be considered as a 'silver bullet' for remediating polluted environments. In choosing a suitable bioremediation method, there are necessary criteria that are considered that include: site of application (in situ or ex situ), depth and degree of pollution, type of the contaminated environment, cost, location and the environmental policies (Frutos et al. 2012; Smith et al., 2015). Generally, it has been found that

autochthonous (indigenous) microorganisms present in a polluted environment play a vital role in solving most of the challenges that are associated with the degradation and detoxification of the pollutants (Verma and Jaiswal, 2016) provided there are suitable environmental conditions for their growth and metabolic processes (Azubuike et al., 2016). For bioremediation to be successfully carried out, the interactions in microbial communities, environmental conditions, physicochemical and structural properties of the contaminants have to be adequately understood (Suthersan and Payne, 2005; Haritash and Kaushik, 2009) these factors can be seen in Figure 1.2. The major advantages of these method are environmental friendliness, cost-effectiveness, can be employed in the treatment of a broad range of contaminants in wastewater and specific treatment of contaminants like petroleum hydrocarbons or metals (Chang et al., 2002). These advantages explain the reasons why bioremediation has gained global acceptance and application in treating a broad range of environmental pollutants compared to other remediation techniques. According to (Evans and Furlong, 2003) bioremediation is divided into in situ and ex situ depending on the location that the remediation process is carried out. The bioremediation process is considered in situ when the process is carried out in the polluted environment. Although this process is carried out in the field where there are limitations to the control of the different variables that can influence the degradation process mostly abiotic factors, the addition of nutrients and electron acceptors is employed to enhance the degradation rate. In situ remediation is mostly used when the pollution is high in-depth, widely spread in a large area and there is a

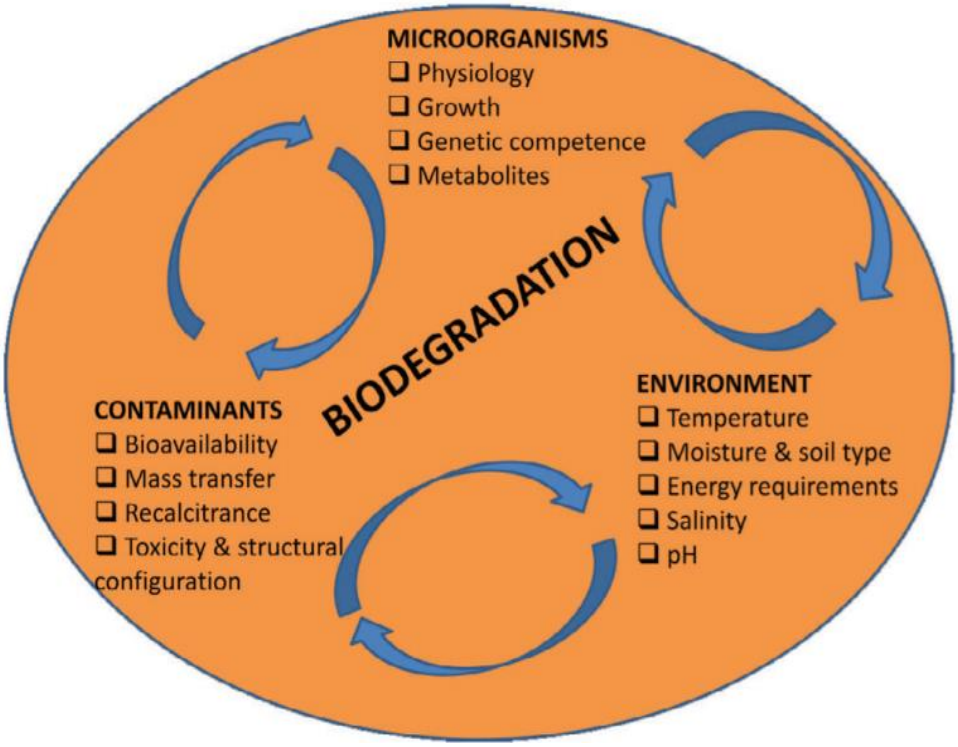


Figure 1.1 Factors affecting bioremediation of contaminants in the environment (Adelaja, 2015)

low concentration of contaminants. On the other hand, ex-situ bioremediation is the physical removal of the contaminated material from its original environment to another environment or facility for treatment. These methods are carried out in an environment that is easily controlled, monitored and will require shorter treatment time. Hence, more efficient than in situ technology. However, ex-situ technologies are both capital and energy-intensive compared to in situ technologies due to excavation of polluted material, the transportation of the polluted material from the site of pollution to the treatment site, and the environmental risks associated with the transportation of hazardous material (Evans and Furlong, 2003). The major techniques that are employed to achieve either in situ or ex situ bioremediation are covered next.

The advantages and disadvantages of each bioremediation method is outlined below in the respective approaches.

1.1.4.2.1 Biopiles

This approach involves the excavation of the polluted soil and pilling it above ground level. The piles are then injected with nutrients and when considered necessary, aeration and moisture (irrigation) are also added with the sole purpose of stimulating the activities of microorganisms degrading the contaminants. A treatment bed and leachate collection systems are also used in this technique. This method is cost-effective and effective when the suitable conditions are provided (Whelan et al., 2015), can help reduce the volatilization of pollutants with low molecular weight, effective in extreme environments like very cold regions (Diaset al., 2015; Gomez and Sartaj, 2014; Whelan et al., 2015) and it requires a smaller space compared to other ex-situ techniques like landfarming. However, the limiting factors to this method are the cost of maintenance and operation, require robust engineering, cannot be used in remote areas where there is no power supply to evenly inject air. Furthermore, the injection of excessive air can cause drying of the soil that is being treated thereby leading to the inhibition of microorganisms' activities and volatilization rather than biodegradation of pollutants (Sanscartier et al., 2009).

1.1.4.2.2 Bioreactors

This involves the use of microorganisms in treating polluted material in a vessel known as a bioreactor. The conditions in a bioreactor are usually controlled to mimic the natural environment of the microorganisms to promote optimum microbial activities that will result in effective biodegradation. The advantages of this approach are its effectiveness, the time for bioremediation is significantly shorter compared to other bioremediation approaches, it is suitable for remediating soil or water contaminated with volatile organic compounds e.g. benzene, toluene, ethylbenzene and xylenes (BTEX) (Mohan et al., 2004), in a crude oil treatment, dynamics in the microbial population can be tracked thereby, enabling the

characterisation of the main microorganisms that are involved in the degradation (Chikere et al., 2012; Zangi-Kotler et al., 2015). Furthermore, substances for bioaugmentation (genetically modified microorganisms) and biostimulation (sewage sludge) can be properly disposed of thereby, preventing any risks associated with these substances when released into the environment (Mustafa et al. 2015). Useful if the target site is highly contaminated or too cold or dry for bacteria to flourish in. However, because the treatment is carried out in a vessel, the quantity of the remediated polluted material is restricted to the capacity of the vessel. Hence, not suitable for larger polluted materials. It is capital intensive, and the risks associated with the transportation of materials polluted with hazardous substances from the site of pollution to the site of treatment (Atlas and Philp, 2005). Lastly, when the variables that are to enhance the process are not properly controlled, this will affect the effectiveness of the process.

1.1.4.2.3 Landfarming

This method involves the excavation and spreading of contaminated soil into layers of not more than 1.5 m thick. These layers are tilled at intervals to ensure adequate aeration and nutrients are also added to enhance the activity of indigenous microbial degraders (Khan et al., 2004). There may be no need for excavation when the pollutant is about 1m below the ground surface but when a pollutant is 1.7 m below the ground surface, it is necessary that the soil is excavated and spread on the ground level for effective biodegradation (Nikolopoulou et al., 2013). The major mechanisms for landfarming are biodegradation and volatilization (Besaltatpour et al., 2011). The advantages of this technique are very simple to design and implement, it is cost-effective, can be used in treating large quantities of polluted soil, has less environmental impact and energy requirement compared to other techniques. (Maila and Colete, 2004). Some of the limitations of this method are extra cost due to

excavation, it requires large operating space and not very effective in remediating soils polluted with inorganic compounds (Khan et al. 2004; Maila and Colete, 2004). Furthermore, this method is not suitable for treating soils contaminated with toxic volatile substances that become toxic aerosols due to one of its remediating mechanisms (volatilization) (Azubuike et al., 2016) this can be a threat to public health when volatilised toxic compounds like benzene is inhaled (WHO, 2000).

1.2 Hydrocarbon pollution in the Niger Delta

The Niger Delta region of Nigeria is one of the most petroleum hydrocarbon polluted region globally. Having suffered recurrent oil spillage for about 5 decades, samples from the region's petroleum hydrocarbon polluted matrices were considered suitable to prospect for microorganisms that are petroleum hydrocarbon degraders. More details on the region and the oil spills are below.

1.2.1 Overview of the Niger-Delta environment and Natural resources.

The Niger Delta is situated in the Atlantic coast of southern Nigeria with an estimated land area of about 70,000 Km^2 (UNEP, 2011). This region has the world's third and fourth largest wetland and mangrove respectively. The Niger Delta houses the largest river delta in Africa that drains Nigeria's two longest rivers (Niger and Benue) into the Atlantic Ocean (Spalding et al., 2010; Könnet, 2014). Its coastal belt of swamps stretches towards the north and becomes a continuous rainforest that gradually merges with central Nigeria's woodland and savannah grassland. The forest, swamp and woodlands are estimated to cover about 12 percent of the total surface area of the region (UNEP, 2011). Considered as one of the key biodiversity hotspots on earth (Ebeku, 2004; Iyayi, 2004 as cited in Okotie et al., 2018), this region was declared as the key zone for the conservation of the western coast of Africa (Zabbey, 2004)

as cited in Chukwuka et al., 2018).). Undoubtedly, it has one of the richest ecosystems that is home to extraordinary biodiversity in the world (Ekeke et al., 2008). Its ecosystem comprises of four ecological zones that include: coastal ridge barrier islands, freshwater swamps, mangrove swamps and lowland rainforests (Ogbe, 2005 as cited in Okotie et al., 2018). In 2016, UNDP reported that in the Niger Delta, there is a high diversity of plant and animal species including those that are locally and globally considered as endangered species. The region has about 46,000 plant species with about 205 being endemic. Unfortunately, 484 species in 112 Families of plants are threatened with extinction (Salau, 1993). It also has approximately 148 species of birds from 38 families and several species of primates (UNDP, 2016). Furthermore, its aquatic ecosystems that are reported to include freshwaters, marine waters and brackish waters are said to support a wide range of fauna (Akankali and Jamabo, 2012). Ekeke et al., in 2008 reported that there are 36 families and 250 species of fish with 20 of the species being endemic. The commonly found fish species include barracuda, finfish, shellfish, tilapia, catfish, denticle and 57 taxa of aquatic insects (Arimoro and Ikomi 2009). On forest resources, the Niger Delta has a large forest reserve with a variety of trees. These include mangrove (*Rhizophora sp*.), mahogany (*Khaya sp*.), iroko (*Milicia excelsa*), abura (*Hallea ledermanmi), Treculia Africana*, *Irvingia gabonensis*, Klainedoxa gabonensis, Albizia adianthi-folia etc (McGinley & Duffy, 2007 as cited in Olarinmoye et al., 2018). Some of the endangered species that can be found in this region include: *Loxodonta africana cyclotis* (Forest Elephants), *Procolobus epieni* (red colobus monkey*), Trichechus senegalensis* (West African Manatee), *Cercopithecus erythrogaster* (whitethroated guenon), *Choeropsis liberensis heslopi* (Pigmy hippopotamus), and *Cercopithecus sclateri* (Sclater's guenon) that are in the IUCN red list of endangered species (UNDP, 2016). Other species that can be found in the Niger-Delta include *Kobus ellipsyprimnu*s (waterbuck), *Limnotragus spekei* (aquatic antelope)

and the endangered *Pan troglodytes vellerosus* (Nigeria-Cameroun chimpanzee) (Chukwukaet al., 2018). Apart from its extraordinary biodiversity, the Niger Delta is also endowed with fertile soil for agriculture and mineral resources such as limestone, marble, barites, sand, gravel and crude oil (Zabbey, 2009; Adelana and Adeosun, 2011; Pegg and Zabbey, 2013, Zabbey et al., 2017). Among these mineral resources from the Niger Delta, crude oil is one of the major resources that has been explored and exploited. With oil production valued over $600b since 1960 (Ite et al., 2013), the extraction of this resource from the Niger Delta for decades has played a significant role in Nigeria's economy and is estimated to account for over 90% and 35% of the nation's total foreign exchange revenue and Gross Domestic Product (GDP) respectively (Akpabio and Akpan, 2010; OPEC, 2015). Despite the extraction of this resource, the country's oil reserve was estimated to still have an export value of $89b per annum (Könnet, 2014; OPEC, 2015). The oil reserve at the end of 2018 was estimated to be 37.5 in thousand million barrels making the country the second highest in Africa and ahead of middle East Qatar and North America Mexico among other countries (BP, 2019). This oil deposit is mostly within the Niger Delta region (Zabbey et al., 2017).

1.2.2 The impacts of petroleum hydrocarbons exploration, exploitation and production on the environment and human health.

Although Nigeria has benefited greatly from the production of oil, unfortunately, its exploration and exploitation in the Niger Delta for decades has negatively impacted the environment and the people living within the region (Kadafa, 2012). Both activities involved in the exploration, exploitation and unplanned incidences have been identified to contribute to these negative impacts in the Niger Delta. These includes: 1. the destruction of large terrestrial habitats for seismic lines and installation of pipelines 2. High levels of soil compaction due to heavy machinery or tillage tools and heavy pedestrian traffic, 3.

Destruction of ecosystems due to gas flaring 4. Alteration of normal growth of living organisms due to vibration, sound and/or noise arising from oil drilling (Chukwuka et al., 2018) 5. Destruction of ecosystems, impact on human health and at some extreme incidences could result in human fatalities due to Oil spillage and/or gas flaring (Kadafa, 2012; Chukwuka et al., 2018). Among these activities and incidences, oil spillage is one of the highest culprits impacting on both humans and the environment. The severity of the impacts can be attributed to the frequency of the occurrence of these incidences and the toxicity of the pollutants. It has been reported that since the discovery of crude oil in the Niger Delta by Shell British Petroleum (now Royal Dutch Shell) in 1956 and subsequent commencement of commercial production in the year 1958 (Onuoha, 2008; Anifowose, 2008), there have been many incidences of oil spills that led to the region being ranked among the top five most severely damaged ecosystems by petroleum hydrocarbons globally. For over 50 years of oil production in the Niger Delta, there have been an estimated 9-13 million barrels of oil spilled into the environment a quantity that is equivalent to 50 times the popular Exxon Valdez spills (Kadafa 2012). Unfortunately, over 77% of the spilled petroleum hydrocarbons were not recovered during this period (Nwilo and Badejo, 2006; Kadafa, 2012). The Nigeria National Oil Spill Detection & Response Agency (NOSDRA) has also recorded 13273 reported incidences of spills that have resulted in an estimated quantity of 698140.61 barrels of crude oil being released into the terrestrial and aquatic environment of the region from 2005 to 2020 (NOSDRA 2020). In 2019, CNN reported that an estimate of over 40 million litres of oil is spilled annually into the environment of the region. These spills have led to the destruction of forests, biodiversity and contamination of rivers and streams leading to the classification of the many known rich ecosystems of the region as ecological wasteland (Kadafa, 2012). One of the most detailed scientific studies conducted in part of the region to assess the level of

contamination was the 2011 Ogoniland assessment that was conducted by UNEP. The result of the studies showed that there have been negative impacts on the environment and human health.

1.2.3 Polluted soil and groundwater.

Both soil and groundwater of the polluted area were found to be contaminated at a level higher than the Environmental Guidelines and Standards for the Petroleum Industries in Nigeria (EGASPIN) as set by the Nigerian National standard. Due to the absence of continuous clay layer across the region, the contaminants reported to have gained access to the groundwater from the surface. In some cases, these contaminants were detected at depths of at least 5m. In an extreme case of groundwater contamination, a layer of 8 cm refined oil was observed to be floating on the groundwater community wells that the people were using as sources of water for domestic activities used (UNEP, 2011).

1.2.4 Human exposure and health implication.

The decades-long pollution shows that the people in Ogoniland had been exposed to chronic oil pollution throughout their lives. These exposures are through drinking water and outdoor air that are sometimes said to be at high concentrations. In one of the communities Nisisioken Ogale, benzene a known carcinogen was detected from their drinking water collected from different wells at levels that were over 800 times above the World Health Organization (WHO) guideline. There were also high concentrations of benzene that were detected in other communities at concentrations that were at least 1,000 times higher than the Nigerian 3 µg/l standard for drinking water. Furthermore, it was reported that residents were exposed to petroleum hydrocarbons through dermal contacts with contaminated sediments, surface water and soil. Although these residents are aware of the danger of being exposed to these contaminants, they had no other alternative rather than use the contaminated water for

drinking, cooking, washing and bathing. About 10 percent of benzene concentrations detected in the studied area were already higher than the concentrations of the United States Environmental Protection Agency (USEPA) and WHO report as a 1 in 10,000 cancer risk equivalent (UNEP, 2011). Benzene is just one of the over 17,000 identified chemical components of crude oil (Daghio et al., 2017). Among these chemical components of crude oil, benzene is grouped with other volatile compounds known as BTEX (benzene, toluene, ethylbenzene) that are known to be harmful to human health. Long exposure of people to these compounds has the potential of causing cardiovascular and respiratory illnesses, affect the development and function of the immune system, reproduction system and metabolisms (WHO, 2000; El-Hashemy and Ali, 2018; Pouresmaeili et al, 2018; Latif et al, 2019). Other impacts include neurological impairment that can be caused by all the BTEX compounds while benzene can increase the risks of cancer it also causes haematological effects such as acute myelogenous leukaemia and aplastic anaemia (WHO, 2000; ATSDR, 2004; Marc et al., 2016). Furthermore, short-time exposure to high concentrations of BTEX may trigger circulatory mortality (Ran et al., 2018). Another group of crude oil known to have negative impacts on human health is the Polycyclic Aromatic Hydrocarbons (PAHs). This group of compounds is of public health concern due to their toxicity, genotoxicity, mutagenicity and/or their carcinogenic characteristics (Ghosal et al., 2016).

1.2.5 Impacts of Petroleum hydrocarbon on vegetation.

From trees to crops in oil-polluted areas are said to be negatively impacted. The mangrove habitat has greatly been damaged to the point that can results to its irreversible loss if nothing is done. For root crops like cassava that grow in polluted areas are not considered safe (UNEP 2011). Other studies have also shown how oil spill affects the germination of seeds, growth of crops and reduces the yield of crops (Chukwuka et al., 2018).

1.2.6 Impacts of Petroleum hydrocarbon on aquatic systems.

Throughout the polluted area, the surface water was either covered with thin sheens or thick black oil. This affects the organisms within these habitats and the ecosystem services they provide. Fish migrate from the polluted areas in search of cleaner water and those within the polluted accumulated hydrocarbons. Hence cannot be considered safe for consumption. The wetlands are said to have deteriorated to the point of facing disintegration (UNEP,2011). The level of environmental deterioration of this region caused by oil spill has resulted in increased poverty and displacement of the people that depended on the now destroyed ecosystems for ecosystems services for their survival (Kadafa, 2012).

1.2.7 Recent approaches for the remediation of the oil-polluted Niger Delta region.

Different remediating approaches have been employed to remediate the polluted area with the most recent remediating project being Hydrocarbon Pollution Remediation Project (HYPREP) which started in 2019. Landfarming and biopile are the approaches that are being piloted by contractors with the intention to scale-up when found effective (HYPREP, 2020). These approaches are both biological methods that are environmentally friendly and are cost-effective compared to chemical and thermal methods. However, when compared to other biological methods, these approaches are expensive and energy-intensive because of the excavation that is associated with them (Evans and Furlong, 2003). According to (Nikolopoulou et al. 2013) for landfarming to be effective, when soil pollution is 1.7 m below the ground surface, it is necessary that the soil is excavated and spread on the ground level. Following the 2011 UNEP assessment, it was reported that there was at least a 5 m depth of petroleum hydrocarbons pollution and there are also reports of many similar polluted sites spread across the Niger Delta region. With the degree of pollution reported, using approaches

such as biopiles and landfarming for the remediation will be very expensive and energy-intensive because of the required manpower and soil excavation. Furthermore, landfarming is not suitable for treating soils contaminated with toxic volatile substances due to one of its remediating mechanisms (volatilization) (Azubuike et al., 2016). Benzene which was detected in high concentrations within Ogoniland (UNEP, 2011), is a toxic and volatile compound that is a threat to public health (WHO, 2000). Landfarming may not be suitable for application within the Niger-Delta region if polluted sites are located within or very close to residential areas. Similarly, when there is excess air injection into biopiles, there will be volatilization rather than biodegradation of pollutants (Sanscartier et al. 2009). Although there are already known limitations for the two approaches, the HYPREP project is still in its infancy and data on the effectiveness of interventions being used is not yet available.

1.3 Problem statement

The challenges faced with the above remediation methods are that either they are slow, expensive, ineffective, energy-intensive and/or not environmentally friendly. This has necessitated the need to develop new technologies and/or improve on existing ones. The European Union in 2019 invested over 10 million Euros for trans-national research to develop new technologies for remediation of environmental contaminants, including hydrocarbons (EU, 2020) indicating the topicality of the problem. Recently, Bioelectrochemical Systems (BES), an emerging biotechnology, has attracted global attention for remediating various contaminants because of its potential effectiveness, low-cost, neutral or positive energy and being environmentally friendly (no or limited need for chemicals) compared to other methods including known biological methods (Wang et al., 2020a). BES technology has shown some promise in remediating different environmental contaminants as recently reviewed by Wang et al., 2020 but challenges remain (section 1.4.1). However, there are limitations that are

associated with the technology that include: 1. the inefficiency in electrons transfer from microbial cells to the solid-phase electrode (Martinez and Alvarez, 2018), 2. Electrode aging during the remediation process reduces the performance of the system, 3. Need to improve the versatility of the strains of bacteria used for BES, 4. Inadequate knowledge on the use of large electrode for in situ applications (Li et al. 2020) and 5. Radius of influence does not extend far from the electrode (Wang et al. 2020a) because pollutant removal depends majorly on electrodes and bacteria interaction (Li et al. 2020).

1.4 Bioelectrochemical systems and bioremediation

Due to the recalcitrant and toxic nature of petroleum hydrocarbons, there have been many efforts to aid in the fast degradation of these pollutants. BES, an emerging technology that facilitates continuous electrons transfer in an optimal working condition of the system, could significantly enhance bioremediation, shorten remediation time and reduce cost (Wang et al., 2020). Bioelectrochemical remediation is a combination of both electrochemical and biological processes that utilize electroactive microorganisms as catalysts for the reduction or oxidation reactions of inorganic or organic electron donors and transfer of electrons to a solid-state anode (Figure 1.3). There are two types of BES namely: Microbial fuel cells (MFC) and Microbial Electrolysis Cells (MECs). A BES is termed as MFCs when the entire reaction of substrate degradation occurs spontaneously resulting in the production of electricity while BES is termed as MEC when the degradation of pollutants occurs simultaneously with the production of hydrogen/methane/water while requiring a small energy input to drive the entire process (Cario et al., 2019).Several studies using this technology for the remediation of petroleum hydrocarbons from sediment, soil and groundwater have recorded various degrees of success. Using BES in Leuna-Germany, a 50 L bench-scale constructed wetland was operated for 400 days and it was found that benzene 12 mg/L and methyl-tert-butyl ether

(MTBE) 3 mg/L were almost completely removed on the 95th and 125th day respectively (Wei et al. 2015). Furthermore, in a 155-day study, Adelaja et al. 2017 using MFCs for the degradation of petroleum hydrocarbons, reported a >90% of degradation efficiency and a simultaneous electricity generation at peak power density of 6.75 mWm^{-2}. Although the technology (BES) is operated under anaerobic conditions, facultative anaerobes such as *Pseudomonas spp*. have been reported to be utilised in the cathodic and anodic chambers of BES during hydrocarbon degradation (Clauwaert et al. 2007; Erable et al. 2010, Adelaja et al., 2015).

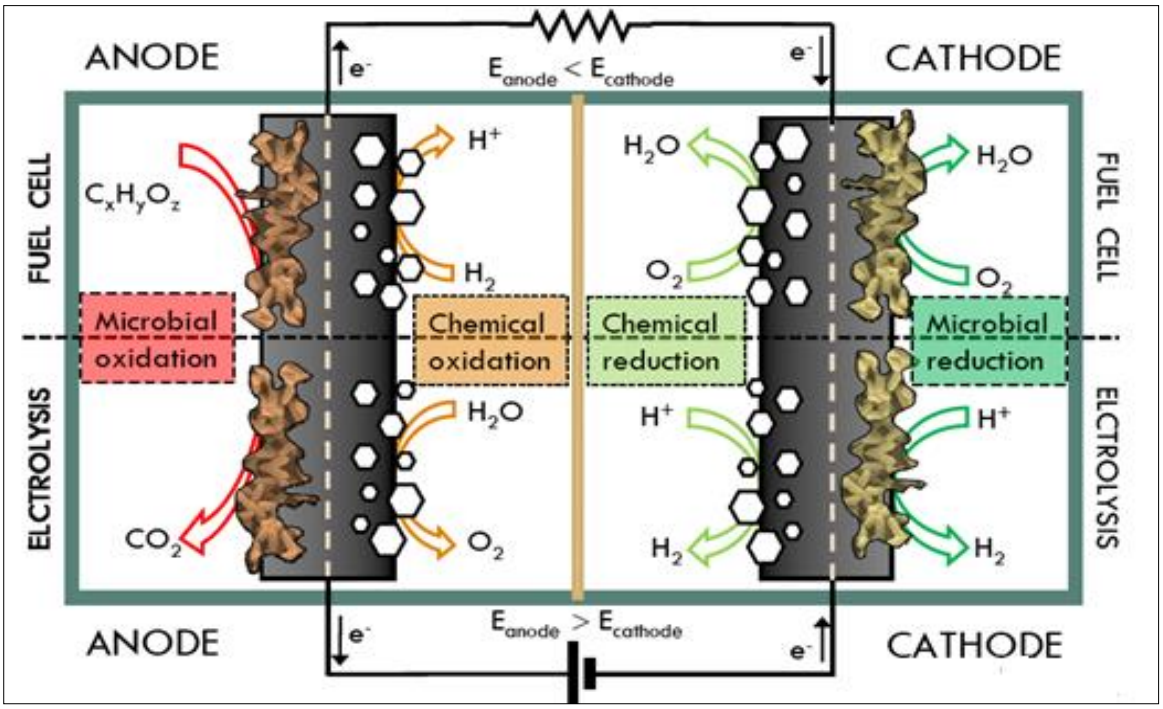


Figure 1.2 The concept behind the operation of bioelectrochemical systems. The upper section of the diagram is Microbial Fuel Cells (MFCs) when there is spontaneous reaction in the system while the lower section is Microbial Electrolysis Cells (MECs) when energy is supplied to facilitate the reaction in the system (Adelaja., 2015)

In MFCs electro active microorganisms oxidise organics at the anode with the electrons released channelled through an external circuit to a cathode where reduction e.g. of oxygen occurs. In MECs, processes in an MFC can be enhanced thermodynamically by addition of a

small voltage, say 200 mV (Fernando, 2014). As electrons are subsequently transferred to a solid-state cathode through an external circuit, energy is generated (Srikanth et al., 2018; Srikanth et al., 2016; Wang et al., 2015). This technology is employed in the remediation of polluted environments due to its capability to continuously accept or donate electrons (Zhang et al., 2013) thereby overcoming the critical limitation that is associated with other bioremediation methods (Liang et al., 2019; Lu et al., 2014; Shen et al., 2014; Wang et al., 2012). This facilitated continuous electrons transfer can significantly enhance bioremediation, shorten remediation time and reduce cost (Wang et al., 2020). BES technology has been utilised for the oxidation or reduction of several pollutants by converting them to either less toxic or more valuable products (Wu et al., 2018). There have been several reports of novel applications of this technology that include but not limited to treatment and remediation of pollutants such as toxic metals and radioactive substances (Gregory and Lovley, 2005; Huang et al., 2011), gaseous biofuels (hydrogen and methane) production (Cheng et al., 2009; Oh and Logan, 2005) synthesis of organic compounds from the reduction of carbon dioxide (Xiang et al., 2017). Recently, this technology has been used for the remediation of groundwater, soil and/or sediments that are contaminated with petroleum hydrocarbons (Zhang et al. 2010; Logan 2009). Figure 1.4 shows a schematic diagram of the technology that involves both sediments and groundwater matrices.

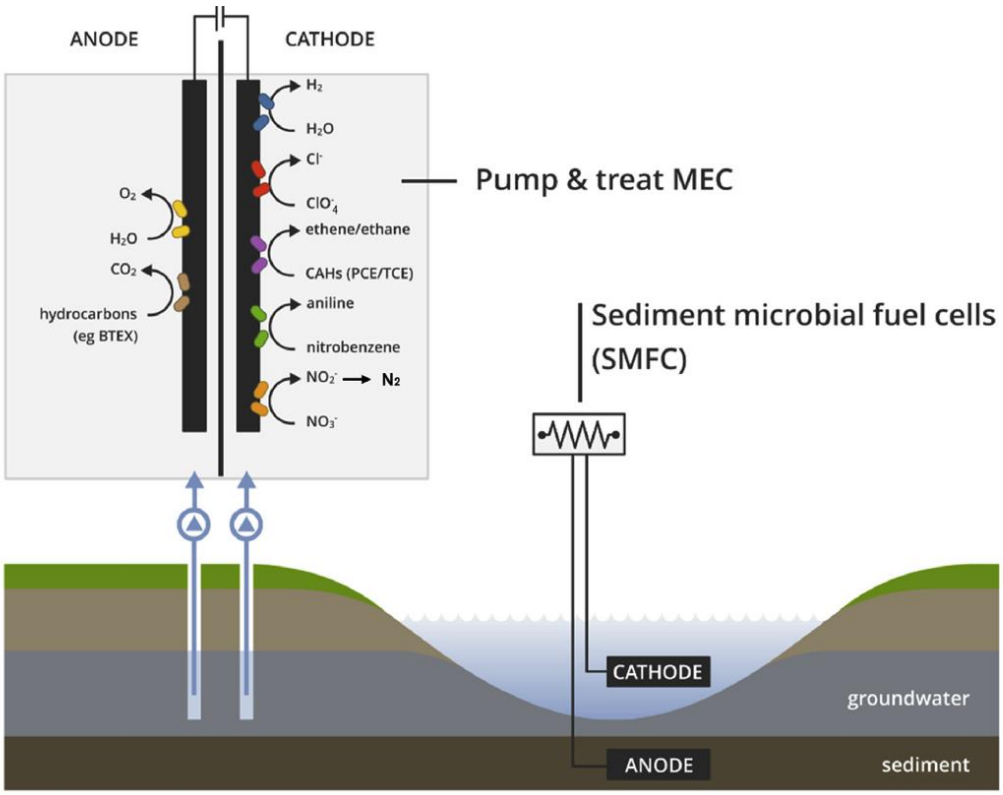


Figure 1.3 Schematic overview of microbial electrochemical remediation methods (Wang et al., 2020b).

It was highlighted that bioelectrochemical remediation is not optimally used due to challenges such as: 1. lack of knowledge on the electroactive microorganisms and their remediation capabilities; 2. Reactor designs that is more suitable to use in a sediment and 3. The effects of supplements such as biochar and surfactant. However, this technology has advantages that include: 1. The capacity of electrodes to provide an unlimited number of electron receptors and donors that facilitate microbial redox reactions. This results in not requiring the use of chemicals (reductants and oxidants) that can cause secondary pollution to the polluted site (Beretta et al., 2019). 2. The costs for BES installation and maintenance is cheaper compared to other methods. 3. Because of its ability to provide both reduction and oxidation transformation, this makes it suitable for removal of a wide range of organic and inorganic pollutants (Wu et al., 2018). 4. It can simultaneously degrade pollutants and generate electricity. Hence, the process of remediation is either energy positive or neutral

and the energy (current) generated can be useful in powering remote sensors. 5. The progress of the degradation can be monitored online. 6. Compared to other methods, the remediation time is shorter (Wang et al., 2020).

1.4.1 Challenges in application of bioelectrochemical systems to bioremediation.

As an emerging technology, the general limitations of BES include poor knowledge on electroactive microorganisms and their contribution to the remediation process (Wang et al., 2020a), diversion of electrons to other electron acceptors such as sulphates and nitrates, overpotential losses at electrodes, and utilisation of expensive materials like platinum. Furthermore, acidification of the anode because of pH gradients from membrane underperformance or mass transfer limitations are among the challenges of BES wide-application (Franks and Nevin, 2010; Rosenbaum and Franks, 2014). To overcome the acidification of the anode, phosphate buffer aimed at maintaining neutral pH are used in liquid systems but unfortunately, this only lasts for short periods. As the buffer ionic strength depletes, protons build up leading to anode acidification that may inhibit the activities of microorganisms or causing overpotential losses at the anode (Pant et al., 2010). In soil systems proton mass transfer limitations affect the radius of influence for degradation of contaminants. Other challenges are non-bioavailability of insoluble petroleum hydrocarbons such as PAHs, bottlenecks in scaling up operations, inadequate knowledge and understanding of the microbiology of microorganisms with regards to electron transfer and the mechanism of extracellular transfer of electrons to the surface of electrode (Pant et al., 2010). There are suggestions that redox mediators may aid the processes of electron transfer (Rosenbaum and Franks, 2014; Clauwaert et al., 2008). In previous studies, bio-treatment of PAHs in wastewater and soil has been carried out using microcosms (Morris and Jin, 2012; Wang et

al., 2012, Lu et al 2014 2017). Bioavailability is a key issue with petroleum hydrocarbons and measures like the use of synthetic non-ionic surfactants e.g. Tween 80 or microorganisms (e.g. *Pseudomonas aeruginosa)* that produce biosurfactants known as rhamnolipids or fungi that produce degradative enzymes could influence the bioavailability and degradation of these compounds. The limitations of BES include: 1. the inefficiency in electrons transfer from microbial cells to the solid-phase electrode (Martinez and Alvarez, 2018), 2. Electrode aging during the remediation process reduces the performance of the system, 3. Need to improve the versatility of the strains of bacteria used for BES, 4. Inadequate knowledge on the use of large electrode for in situ applications (Li et al. 2020) and 5. Radius of influence does not extend far from the electrode (Wang et al. 2020a) because pollutant removal depends majorly on electrodes and bacteria (Li et al. 2020).

1.4.2 Potential strategies of enhancing bioelectrochemical remediation of petroleum hydrocarbon

1.4.2.1 Surfactants

This rise in concentration of pollutants can be said to be attributed to the pollutants' molecules that absorb to the polluted matrix (O'Connor et al., 2018) and the non-bioavailability of these pollutants for biodegradation (Adelaja, 2015). These have necessitated to the wide application of surfactants to enhance the remediation of petroleum hydrocarbon polluted matrices in other to enhance the process (Davin et al., 2018; Pei et al., 2017; Alcantara et al., 2009; Lai et al., 2009). Surfactants are said to play a key role in facilitating the desorption of solid phase petroleum hydrocarbon pollutants and enhancing the dissolution of nonaqueous phase liquids by reducing the oil/water interfacial tension and the air/water surface tension of solution (Wei et al., 2020; Zhou et al., 2011). In insoluble substrates, surfactants are said to also play key roles by enhancing the interaction between insoluble

substrates and microorganisms that helps in facilitating substrate transport to the cell (Kavitha et al., 2014; Varjani and Upasani, 2016). Surfactants hold enormous potential in enhancing the degradation of petroleum hydrocarbon pollutants in the environment. Therefore, prospecting for biologically based surfactants and integrating them to BES may enhance the degradation of petroleum hydrocarbon pollutant.

1.4.2.2 Electrode design

There have been different MFC electrode designs made in an effort towards optimising the MFC system for pollutants degradation. One of these electrode designs is a snorkel. A snorkel is one of the simplest MFC designed with a direct coupling of both the anode and the cathode (Erable et al., 2011). This can simply be described as a short circuit MFC and when short-circuited there is zero voltage between the anode and the cathode thereby leading to no power but works at the possible maximum current an MFC can produce. Because of its ability of sustaining maximum current between the cathode and the anode, the system can achieve a maximum reaction rate according to its capacity, thereby making the snorkel suitable for raising electrochemical reaction rate that is aimed at pollutant removal rather than production of electricity (Santoro et al., 2017). The use of snorkel has been reported to be effective in the remediation of petroleum hydrocarbon (Viggi et al., 2015; Matturro et al., 2017). Another MFC electrode design is air breathing. This type of electrode design does not have cathode and anode coupling but has a cathode with a diffusion layer that controls oxygen diffusion from the air to the cathode thereby removing external aeration demand (Liu et al., 2015). In air breathing MFC, the cathode has direct contact with the electrolyte on one side and on the other side, also has direct contact with air (Vogl et al., 2016). Comparing these two different designs for the remediation of petroleum hydrocarbon can provide an insight of which is most suitable in enhancing the degradation of the pollutants

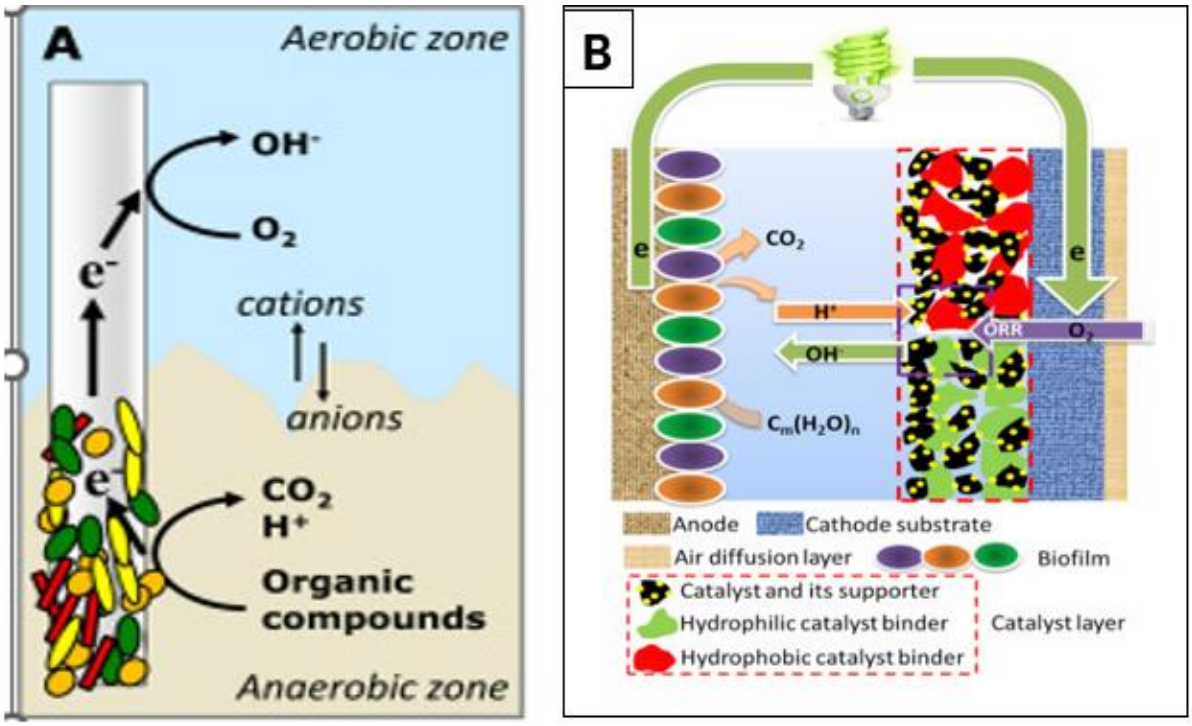


Figure 1.5 (A) Schematic design of snorkel microbial fuel cell (Morgane et al., 2019), (B) Schematic design of air breathing microbial fuel cell (Zejie et al., 2017)

1.4.2.3 Biochar

Biochar is a carbon rich material that is produced by the converting carbonaceous compound or biomass at high temperatures between 600^{0}C and 800^{0}C in the absence of oxygen (Gupta et al., 2020). Biochar as an energy effective carbon material has microporosity, high ion exchange potential and a large surface area. Its microporosity characteristics has given this material the potential of its suitability in environmental application due to abundant feedstock and low cost (Ahmad et al., 2014; He et al., 2022). Biochar has been applied in remediating soil from organic contaminants and heavy metals. This is due to its ability to effectively adsorbs these contaminants from the soil. Biochar adsorption of pollutants is said to occur through interactions that include acid–base interaction, hydrogen binding, surface complexation, π-π interaction and electrostatic attraction (Zhang et al., 2013). Biochar has

been reported to enhance the degradation of petroleum hydrocarbon by increasing the relative abundance of PAHs degraders thereby resulting in increase in PAHs degradation in soils (Kong et al., 2018). Zhang et al. (2020) reported an increase in PAHs biodegradation rate in soils with biochar compared to the soils without biochar. These and several studies have reported the positive effects of biochar in the biodegradation of petroleum hydrocarbons. Recently, biochar has also found application in microbial fuel cell as electrode or as catalyst of the system for contaminants remediation and power generation (Saran et al., 2023). In MFC, biochar plays a key role in transporting electrons through electron shuttles and electrical conductivity, thereby enhancing the MFC system by increasing the scope and efficiency of microorganisms' metabolism (Shi et al., 2019). Owing to roles of biochar in MFC, the effect of biochar on the radius of influence will be assess in this research.

1.4.3 Overall aim

To develop and test bioelectrochemical system-based petroleum hydrocarbon remediation technologies at laboratory scale in environmentally relevant conditions (with a view to field deployment).

1.4.4 Hypothesis

Electrodes inserted in soil/groundwater/sediment could increase oxidant delivery to support anaerobic biodegradation of petroleum hydrocarbons in soils, sediments and groundwater speeding up bioremediation and minimising/eliminating energy and chemicals need.

1.4.5 Specific objectives:

1. **To ascertain the characteristics (physicochemical factors as well as microbial composition) of samples from selected petroleum hydrocarbon polluted matrices (soil, sediment and groundwater) from the Niger-Delta region of Nigeria.**

It is well known that many petroleum hydrocarbon-degrading bacteria can achieve excellent results during degradation of petroleum hydrocarbons under laboratory conditions yet exhibit unsatisfactory results in field-scale tests. While it is difficult and nearly impossible to replicate the entire environmental factors (biotic and abiotic) of a polluted site in the laboratory, having an insight on the physicochemical properties and microbial community of these sites can help in designing laboratory experiments. These information on the polluted sites can be useful in designing laboratory experiments using environmentally relevant conditions that are closest to the polluted matrices.

2. **To constitute microbial consortia (microcosms) from autochthonous microorganisms from petroleum hydrocarbon polluted environments for accelerated biodegradation/ biotransformation of the targeted petroleum hydrocarbon pollutants**.

   These consortia will be constituted using different pure strains that will be prospected and selected from petroleum hydrocarbon polluted samples using different screening pressures (benzene as the sole carbon source and manganese IV oxide and iron III oxide as electron acceptors).

3. **To investigate the influence of different surfactants on biodegradation of petroleum hydrocarbon pollutants (phenanthrene) in MFC**

   Two different surfactants (chemical and biological), negative control and microcosm alone will be investigated on their influence in the degradation of petroleum hydrocarbon especially phenanthrene that is a recalcitrant pollutant that is not readily available for biodegradation. The chemical surfactant (Tween 80) and a biological surfactant will be used.

4. **To assess the impact of biochar supplements on the radius of influence in the degradation of petroleum hydrocarbons in a soil MFC experiments at 4 cm and 8 cm distances from the electrode.**

   Biochar will be use as a supplement in soil MFC with the aim of assessing its impact on the radius of influence of an electrode. Using loam soil that is composed of the different types of soil (sandy, silt and clay), the impacts of biochar in enhancing the degradation of the PH pollutants will be investigated at different distance from the electrode (4 cm and 8 cm).

5. **To assess the effects of different MFCs configuration (snorkel reactor and the air breathing reactor) in the degradation of petroleum hydrocarbon pollutants**.

   Each of the MFC configuration (snorkel and air breathing) will be use in a bioreactor that mimics a sediment matrix to ascertain which of the configuration will enhance the degradation off petroleum hydrocarbon better.

Chapter 2: Materials and Methods

Overview

This chapter contains the experimental design, methods and materials used for this research. This include but is not limited to the 1. physicochemical properties of petroleum hydrocarbon polluted samples used for the isolation of microorganisms for microcosms that were used for the degradation of target contaminants (benzene and phenanthrene) 2. Methods used for prospecting the microcosms 3. Method used for the identification of petroleum hydrocarbon degraders and the bacteria communities in the polluted samples 4. Integration of microcosms with different MFCs configuration and 5. The integration of supplement (surfactant and biochar), microcosms and MFC for the degradation of petroleum hydrocarbon.

The targeted petroleum hydrocarbon pollutants for remediation used for this research are Benzene a representative of the BTEX (Benzene, Toluene, Ethylbenzene and Xylene) compounds and phenanthrene a representative of Polycyclic Aromatic Hydrocarbon (PAH). The toxicity of these two compounds makes these two compounds being appropriate for use as target/model contaminants for remediation.

The research proposed was based on literature and previous findings by Adelaja (2015) and the methods used for this research were adapted from these.

2.2 Sampling and physicochemical characterization of petroleum hydrocarbon polluted matrices

Contaminated petroleum hydrocarbon samples (soils/sediments/groundwater) were collected from River State of the Niger-Delta region of Nigeria. These samples were individually characterised for their physiochemical properties. Physicochemical analysis of the samples was carried out by i2 Analytical Ltd, 7 Woodshots Meadow, Croxley Green Business Park Watford, United Kingdom.

2.1 Flow diagram of the experimental design for the research

The flow diagram (Figure 2.1) below shows the step-by-step experiments that were carried out in this research.

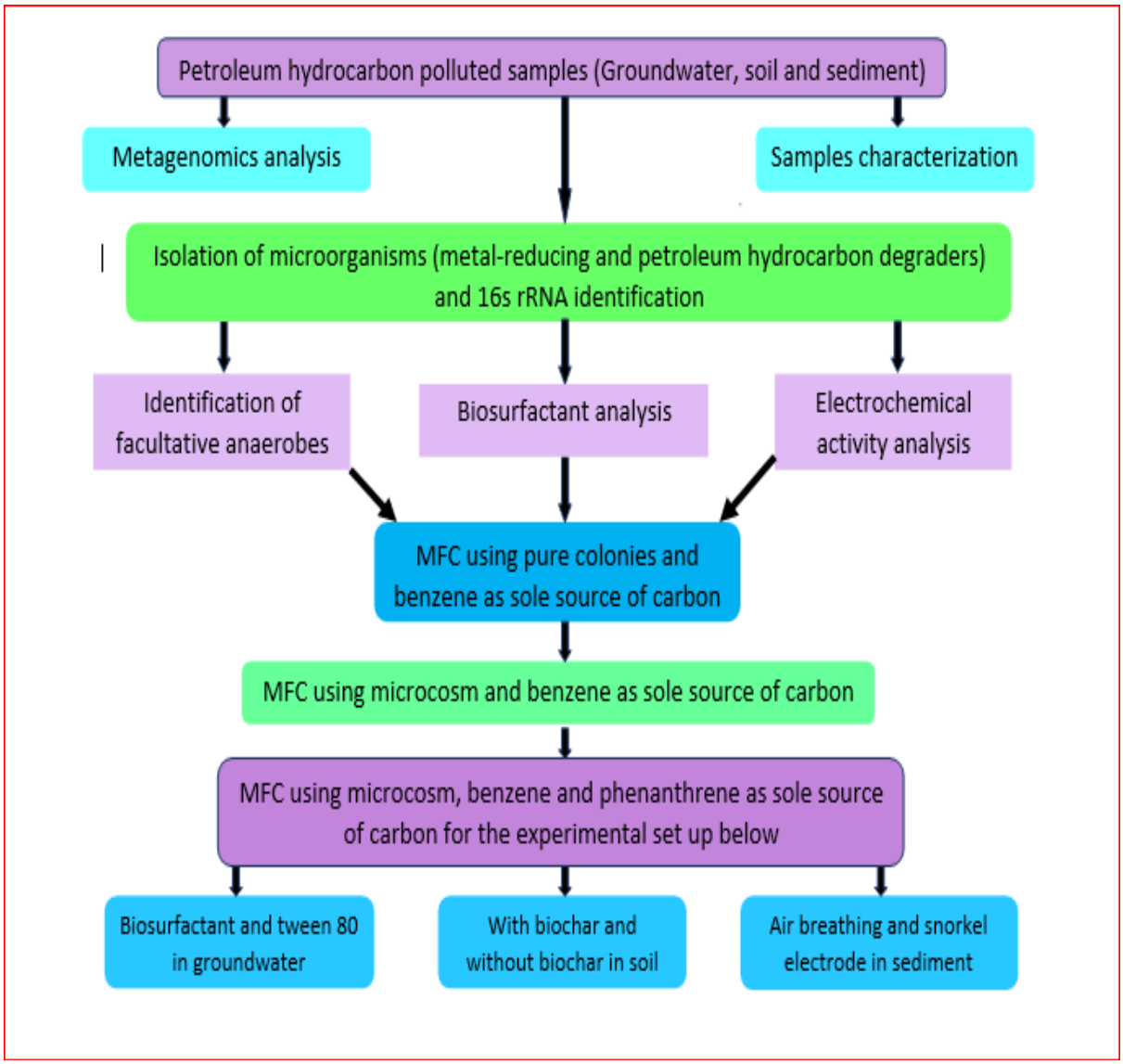


Figure 2.1 A flow diagram showing a step-by-step experiment designed for this research.

2.3 Prospecting for petroleum hydrocarbon-degrading, electrochemically-active and bioemulsifier and/or biosurfactant producing microorganisms

The petroleum hydrocarbon polluted samples in section 2.2 were used as a starting point to isolate facultative anaerobic petroleum hydrocarbon-degraders that are electrochemically-

active and may produce biosurfactant or bioemulsfier. Two different approaches were used namely general medium to enrichment medium method (section 2.3.2) and enrichment medium to general medium method (section 2.3.3) to isolate microorganisms with these characteristics that has the potential to facilitate faster degradation of petroleum hydrocarbons. The selection pressures used were petroleum hydrocarbon (benzene) as the only carbon source while manganese IV oxide and iron III oxide were used separately as the electron acceptors. These microorganisms were selected based on the characteristics that include: 1. Ability to degrade petroleum hydrocarbons (utilise benzene as the sole source of carbon) 2. Good electrochemical activity 3. They are facultative anaerobes and lastly 4. Ability to produce bioemulsifier/biosurfactant an important but not a mandatory characteristic.

2.3.1 Preparation of enrichment media composition

The enrichment media for the isolation of petroleum hydrocarbon degraders with electrochemical activity potential was prepared using the composition below.

Materials

The materials used were as follows:

Defined Minimal Salt Medium (MSM)

2g/L of benzene stock solution.

Stock of 20 mM each of Iron III oxide and Manganese IV oxide.

20mls Sterile universal bottles

Pure bacterial colonies

2.3.1.1 Minimal Salt Medium (MSM) preparation

The defined minimal medium for bioelectrochemical experiments was prepared as follows (per litre 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 in in Tables 2.1 and 2.2.

Table 2.1 100x Trace element solution

Component	Concentration (mg/L)
Nitrilotriacetic acid (NTA)	1500
$MnCl_2.4H_2O$	100
$FeSO_4.7H_2O$	300
$CoCl_2.6H_2O$	170
$ZnCl_2$	170
$CuSO_4.5H_2O$	40
$AlK(SO_4)_2.12H_2O$	5
H_3BO_4	5
$NaMoO_4$	90
$NiCl_2$	120
$NaWO_4.2H_2O$	20
$NaSeO_4$	100

Table 2.2 100x Vitamin Mix solution

Component	Concentration (mg/L)
P-aminobenzoic acid (PABA)	50
L-Ascorbic acid	100
Folic acid	50
Riboflavin	10
Nicotinic acid	100
Panthotenic acid	100
Thiamine hydrochloride	10
Biotin	100

2.3.1.2 Benzene stock solution preparation.

To prepare the benzene stock, a litre of deionised water was sterilised using the autoclaved 121^0C for 15 minutes and was allowed to cool. To prevent the volatilization of benzene a known volatile compound during autoclaving, it was filtered into the cooled deionized water. Using a syringe, 2g of 99.8% benzene was filtered using a Millex-GV Syringe Filter Unit, 0.22

µm, PVDF, 33 mm, gamma sterilized from Sigma Alrich into the litre of sterilised deionize water to obtain the stock solution of 2 g/L.

2.3.1.3 Preparation of electron acceptors – Iron III Oxide and Manganese IV Oxide.

To prepare 20 mM of Iron III Oxide and Manganese IV Oxide, 1740 mg of manganese IV oxide and 3190 mg of Iron III oxide were weighed and each separately transferred into 1 L of deionised water. These were then autoclaved at 121^0C for 15 minutes and were allowed to cool to room temperature.

2.3.2 General medium to enrichment medium method

This method involved the inoculation of the petroleum polluted samples onto a general bacteria growth medium. General purpose media often called general media are media that contain enough nutrients that support the growth of a wide range of microorganisms. Owing to the fact that environmental samples are usually expected to contain many microorganisms whose characteristics are unknown, there are chances of having microorganisms that may release toxins that may inhibit the growth of other microorganisms of interest. Although there are several general media, Mueller Hinton Agar (MHA) was the preferred medium because of its starch content that absorbs toxins released by microorganisms and because it supports the growth of many different types of bacteria. This characteristic makes MHA the most suitable for the environmental samples which may contain bacteria that release toxins capable of interfering with the growth of microorganisms of interest. The microorganisms grown were subsequently sub-cultured by streak-plating on MHA to obtain pure colonies. The Pure colonies that were isolated from this medium were then subjected to a screening pressure using an enrichment medium containing 200 mg/L of benzene as the sole carbon source as previously used by Adelaja (2015) and 20 mM of either iron III oxide or Manganese IV oxide

as electron acceptor to ascertain their potential to degrade petroleum hydrocarbon (benzene) and to also utilise either iron III oxide or Manganese IV oxide as electron acceptor. Details of the steps used in the "**General medium to enrichment medium method"** are shown below**.**

2.3.2.1 Serial dilution of petroleum hydrocarbon polluted soil and sediment sample.

10g of the polluted soil sample was weighed and added to a bottle containing 90 mL of sterile deionized water. This was shaken thoroughly to ensure an even distribution of the contents in bottles. Before the heavier particles settled, 1 mL of the suspension was taken using a sterile pipette and was transferred into a bottle containing 9 mL sterile deionized water and the bottle was labelled soil 10^{-1}. After the bottle was thoroughly mixed by shaking, 1 mL was taken using a sterile pipette and was transferred into another bottle containing 9 mL sterile deionized water and the bottles was labelled soil 10^{-2}. This procedure was repeated for the sediment sample.

2.3.2.2 Serial dilution of petroleum hydrocarbon polluted groundwater sample

1ml of the polluted groundwater sample was taken and added to a bottle containing 9 mL of sterile deionized water. This was shaken thoroughly to ensure an even distribution of the sample. Using a sterile pipette, 1 mL of the diluted sample was transferred into another bottle containing 9 mL of sterile deionized water and the bottle was labelled 10^{-1}. After the bottle was thoroughly mixed the diluted sample, using a sterile pipette, 1 mL was transferred into another bottle containing 9 mL sterile deionized water and the bottles was labelled soil 10^{-2}.

2.3.2.3 Spread plating of the polluted environmental samples

To grow bacterial colonies, three pre-prepared Muller Hinton's agar plates were labelled soil, sediment and groundwater and a duplicate of each was made. Using a sterile pipette, 0.1 mL was taken from the soil dilution factor 10^{-2} onto a labelled plate. Using a sterile plastic spreader, while holding the lid of plate close by the drop of inoculum was spread all over the surface of the plate. To ensure more evenly distribution, the quadrant spread approach was used until there was no traces of free liquid and the lid was replaced. This was done under aseptic condition and as quickly as possible to prevent contamination of the agar with airborne microorganisms. The plates were incubated at 28^0C under aerobic condition for 2 days. To grow the anaerobic bacteria, the spread plate was repeated, and the plates were inverted and placed in anaerobic jars. Subsequently, an anaerobic generating sachet (Oxoid™ AnaeroGen™ 2.5L Sachet) from Thermofisher Scientific was put into each jar and the lid of the jar was immediately closed. All these were carried out under aseptic condition. The jar containing the plate was then incubated at 28^0C for 7 days.

2.3.2.3 Isolation and screening of pure colonies

To isolate pure colonies from both the aerobic and anaerobic plates, individual bacterial colonies that looked morphologically distinct from one another were identified and marked. To obtain only an individual colony (pure colony) on a plate, a wire loop was flamed on a bunsen burner until the loop was red hot. This was cooled by touching a bare spot on the plate of interest. Using the sterile wire loop, a small amount of each of the distinct colonies was taken onto the loop and streaked on a separate pre-labelled Muller Hinton agar plate. Subsequently, an anaerobic generating sachet (Oxoid™ AnaeroGen™ 2.5L Sachet) from Thermofisher Scientific was put into the jar and the lid of the jar was immediately closed. All these were carried out under aseptic condition. The jar containing the plate was then incubated at 28^0C for 7 days.

2.3.2.4 Screening of pure isolates using enrichment medium

The isolated pure colonies (section **2.3.2.3)** were then subjected to a screening pressure that promoted the growth of petroleum hydrocarbon degraders and with electrochemical activity potential. The total targeted volume for the enrichment medium was 20 mls. 16 mls of the already prepared MSM was transferred into sterile universal bottles. 2mls of the already prepared 2g/L of benzene stock solution was added to the MSM, followed by 2mls iron III oxide and manganese IV oxide in separate bottles. The final 20mls of the medium contained 20 mM of either iron III oxide or manganese IV oxide as the electron acceptor and 200mg/L of benzene as previously used by Myers and Nealson, 1988 and Adelaja, 2017 respectively. Both the carbon source and the electron acceptors constituted 10% each of the total volume. Using a sterile wire loop, a loopful of each of the isolated pure colonies was taken and inoculated into the enrichment medium and was duplicated. The control contained only the enrichment medium without inoculating any microorganism. To create an anaerobic condition for the inoculated organisms, nitrogen gas was passed into the bottles for at least 1 minutes to degas oxygen from the bottles. The lids of the bottles were closed and wrapped with parafilm immediately. All these procedures were carried out under aseptic conditions. The bottles were then incubated in a sharker at 28^0C and 150RPM for 30 days.

2.3.3 Enrichment medium to general medium method

This method involved the inoculation of the polluted environmental samples into the enrichment medium. Enrichment media are conditioned to promote the growth of the organisms of interest over others. The enrichment culture is usually designed by introducing certain nutritional growth requirements or manipulating environmental conditions primarily to promotes the growth of the organism of interest. In a situation where the desired organisms are in a small number, enrichment cultures are said to increase these organisms to

detectable levels (Bari & Yeasmin, 2021). Since the microorganisms of interest are expected to be petroleum degrading and electroactive organisms, benzene was used as a sole source of carbon and either iron III oxide or manganese IV oxide were used separately as electron acceptors to serve as the selection pressures.

2.3.3.1 Composition of enrichment culture, inoculation and the incubation of environmental samples

The MSM, stock solution for benzene and the electron acceptors were prepared as initially done in the different sections in section 2.3.1 above.

The composition of enrichment medium was constituted as follows:

14 ml of the MSM was transferred into sterile universal bottles. 2 ml of the already prepared 2 g/L of benzene stock solution was added to the MSM, followed by 2mls of Iron III oxide and Manganese IV oxide in separate bottles. The total volume of the 20 ml of the medium contained 20 mM of either Iron III oxide or Manganese IV oxide as the electron acceptor and 200 mg/L of benzene as previously used by Myer and Nealson (1988) and Adelaja (2017) respectively. Using a sterile micro pipette tip, 2 ml from the dilution factor of 10^{-2} in 2.3.1 of each polluted environmental sample (groundwater, sediment and soil) was taken and inoculated into the enrichment medium. 2 ml of sterile deionised water was used for the negative control instead of the environmental samples. The inoculation was done in duplicate for each environmental sample with benzene being the sole source of carbon and iron III oxide and manganese IV oxide each being an electron acceptor. To create an anaerobic condition for the microorganisms in the inoculated samples, nitrogen gas was passed into the bottles for at least 1 minute to degas oxygen from the bottles. The lids of the bottles were closed and wrapped with parafilm immediately. All these procedures were carried out under aseptic conditions. The bottles were then incubated in a shaker at 28^0C and 150 RPM. After 7 days of

incubation, bottles of the enrichment medium inoculated with the polluted groundwater samples showed a significant clearance of red-brownish and black colour of iron III oxide and manganese IV oxide respectively. This gave an indication of the presence of organisms that are benzene degraders and may be electrochemically active. Using a sterile pipette, 0.1 mL was taken from the bottles and was transferred to already prepared MHA. A sterile plastic spreader was used to spread the drop of inoculum over the surface of the plate. To ensure more even distribution, the quadrant spread approach was used until there was no traces of free liquid and the lid that was held close all through was used to cover the plate immediately. This was done under aseptic condition and as quickly as possible to prevent contamination of the agar with airborne microorganisms. Due to the turbidity of the soil and sediment samples, ascertaining the level of colour disappearance was difficult. HPLC was used to measure the benzene concentration of these samples. Showing no peak for benzene, these samples were also spread plated on MHA like the groundwater. The plates were inverted and placed in an anaerobic jar and an anaerobic generating sachet (Oxoid™ AnaeroGen™ 2.5L Sachet) from Thermofisher Scientific was put into the jar and the lid of the jar was immediately closed. All these were carried out under aseptic condition. The jar containing the plates was then incubated at 28^0C for 7 days. Going forward, these microorganisms were identified. The enrichment media containing the polluted samples (soil and sediment) and the negative control were analyzed by high-performance liquid chromatography (HPLC, Dionex GS50, USA) using a Photo-diode Array (PDA) detector (DIONEX, PDA-100) at 254 nm to ascertain whether there was a reduction in the concentration of benzene. The reduction in the concentration of benzene is a key indicator of the presence of benzene degraders. The injected volume was 20 µL with a column oven temperature of 25°C and the HPLC was operated at isocratic conditions. A reverse phase column, Supelcosil™ LC-PAH column 15cm × 4.6 mm, 5µm was

used. The mobile phase was 50 % acetonitrile and 50 % high purity HPLC water with a flow rate of 0.6 $mLmin^{-1}$. The minimum detectable concentration for benzene and phenanthrene was previously determined to be 50 μgL^{-1} and 5 μgL^{-1} respectively (Adelaja, 2015).

2.3.4 Identification of isolated pure strains using 16S rRNA

All the pure strains of microorganisms that were isolated from the two culture-based methods in sections 2.3.2 and 2.3.3 were identified using 16S rRNA. This was aimed at identifying and excluding any potential pathogen and to also understand the different strains of microorganisms that are petroleum hydrocarbon degraders, anaerobes and may have good electrochemical activity and are biosurfactants producers. To ensure that the microorganisms isolated are facultative anaerobes, all were grown on Mueller Hinton Agar at 28^0C for 7 days under anaerobic conditions.

The procedures below were carried out towards the identification of the microorganisms.

2.3.4.1.DNA Extraction

The DNA of the pure colony was extracted using InstaGene (Biorad 732-6030, Hercules, CA, USA) according to the manufacturer's instructions.

From the pure colonies grown, one large isolated bacterial colony was picked while at least 2 colonies were picked for small colonies and resuspended in a 1 ml of sterile high purity water in a 1.5 ml centrifuge tube. This was centrifuged for 1 min at 12,000 rpm. The supernatant was discarded by carefully using a 1000 µl sterile pipette to withdraw the supernatant without tampering with the pellet. 100 µl of InstaGene matrix was added to the pellet and was incubated at 56^0C on a heat block for 30 min. The tubes were shaken for 10 sec on a vortex and were incubated again on the heating block at 100ºC for 8 mins. These tubes were then shaken for 10 sec and subsequently at 12,000 rpm for 3 min. 70µl of the upper layer

(supernatant) containing the DNA template was then transferred to a sterile Eppendorftube. The concentration and purity of DNA was then determined using a Nanodrop and samples were stored in a freezer afterward.

2.3.4.2 PCR for the extracted isolates' DNA

Primers, based on conserved regions of the 16S rRNA gene, were used to direct PCR amplification of a 940 bp portion of the gene. The primers used were:

(a) 27F primer AGAGTTTGATCMTGGCTCAG

(b) 1492R primer GGTTACCTTGTTACGACTT

Table 2.3 below are the reagents that were used for the PCR. **All reagents were kept on ice**

Table 2.3 The PCR reaction mixture of reagents and their respective volume used for 16S rRNA gene region

Reagent	For 1 sample (µl)
Water (autoclaved distilled)	36.75
10 x PCR buffer (with $MgCl_2$)	5.00
dNTP (1.25mM)	5.00
Primer 27F (100µM)	0.50
Primer 1492R (100µM)	0.50
Taq DNA polymerase (5U)	0.25
Chromosomal DNA	2.00
Total	50.00

For the multiple DNA samples, the PCR reagents mix was prepared for the total number of samples and +2 to make up for some of the mix that may adsorb on the tube surface. This implies that when there were 5 samples for the reaction, a combined reaction mixture was prepared in a tube as in **Table 2.3** for 7 samples apart from the chromosomal DNA. 2 µl of

chromosomal DNA of each sample was transferred into a sterile PCR tube. 48 µl of the prepared combined reaction mixture was then transferred to each PCR tube containing the 2 µl of chromosomal DNA. For the negative control, 2 µl of sterile high purity water was transferred into a sterile PCR tube followed by the addition of 48 µl of the prepared combined reaction mixture. It is important to note that because of the minute quantity of 2 µl of chromosomal DNA it was put on the side of the PCR tube where it can be confirmed that it was usually transferred into the tube. The PCR tubes were then transferred to the PCR machine (DNA thermal cycler) and the amplification of DNA was performed under the following conditions:

Initial denaturation - 5 min at 95°C

35 cycles of 1 min at 94°C (Denaturation)

1 min at 55°C (Annealing)

1 min at 72°C (Elongation)

5 min at 72°C (Extension)

2.3.4.3 Gel Electrophoresis.

Gel electrophoresis was carried out for the amplified PCR products for the 16s rRNA. The procedure used was as follows:

50 mls of Tris/Borate/EDTA (TBE) buffer was diluted in 450 mls of water. To make the gel, 1.5g of agarose was measured and added to 100 mls of the diluted TBE solution. This was followed by the addition of 10 µl of SYBR safe. This mixture was then heated close to boiling in the microwave until the then turbid solution was seen to be clearer. This was allowed to cool to a lukewarm temperature. To cast the gel unto a tray, a ladder was placed on the tray followed

by the pouring of the lukewarm gel. After cooling and solidifying, the ladder was carefully removed and the tray containing the gel was transferred to a tank (gel box). The diluted TBE buffer was poured into the tank until it reached the surface of the gel. To prepare the samples (PCR products) for loading, 5 µl of each was mixed with 2 µl of loading dye. 5 µl of DNA ladder was loaded on the first well of the gel followed by 5 µl of the prepared samples in subsequent wells. The channels were then connected to a power source and ran at 100 V for about 30 minutes. The gel was then transferred to an ultra-violet (UV) transilluminator for the visualisation of the DNA fragments.

2.3.4.4 Purification of PCR products and 16s rRNA sequencing

The PCR products were purified using a QIAquick PCR purification Kit (Qiagen) and according to the manufacturer's instructions.

- 96–100% ethanol was added to Buffer PE before use (the volume ratio was as stated on the bottle label).
- All centrifugation steps were carried out at 132000 rpm in a conventional table-top microcentrifuge at room temperature.

Purification procedure

225 µl of buffer PB was mixed with 45 µl of the PCR products in a sterile Eppendorf tube. A QIAquick column was placed in a 2 ml collection tube. To bind the DNA, the sample mixed with buffer PB was transferred into the QIAquick column that was already placed in the provided 2 ml collection tube. This was centrifuged for 60 secs and the flow-through was discarded and the QIAquick column was placed back in the same collection tube. To wash, 750 µl Buffer PE was added to the QIAquick column centrifuge for 60 secs. The flow-through was discarded and the QIAquick column was placed back into the same tube. This was once

more centrifuged for 1 min to remove residual wash buffer. Each QIAquick column was then removed from the collection tube and placed in a clean sterile 1.5 ml microcentrifuge tube. To elute the DNA, 30 µl Buffer EB was added to the center of the QIAquick membrane and the column was allowed to stand for 1 min and then centrifuged and the purified DNA was immediately stored in the freezer. Prepared based on their specification, the purified DNA fragments were sent to Genewiz for 16s RNA using sanger sequencing. The sequence generated for each colony across the three matrices were submitted to the nucleotide blast of National Centre for Biotechnology Information (NCBI) database website by using the highly similar sequences (megablast) of the data base. The highly similar organisms recovered from the NCBI megablast were submitted to the NCBI taxonomy browser to identify the taxonomy of each of the highly similar organism to the isolates. The various level of taxonomy of each pure colony and the percentage abundance of species belonging to phyla present were ascertained for each of the two culture-based approaches across the different matrices.

2.3.5 Cyclic voltammetry for the assessment of pure isolates electrochemical capacity.

A Cyclic Voltammetry (CV) test involves the scan of a range of potential voltages while measuring current. In the CV experiment, the potential of an immersed, stationary electrode is scanned from a predetermined starting potential to a final value (called the switching potential) and then the reverse scan is obtained. This gives a 'cyclic' sweep of potentials and the current vs. potential curve derived from the data is called a cyclic voltammogram**.** Cyclic voltammetry can yield information regarding the stability of transition metal oxidation state in the complexed form, reversibility of electron transfer reactions, and information regarding reactivity (Goel et. Al., 2021). The electroactivity of enrichment microbes was tested according to the method by Mani 2019. 48 hours prior to experimental set up, each of the

pure colonies was sub-cultured on a nutrient agar plate. Carbon cloth electrodes was measuring 6 cm (2 cm X 3 cm) was cut and an electrical wire measuring approximately 20 cm soldered to it. A small hole was bore at the centre of 45 ml falcon tubes lids. Each of the soldered electrodes was transferred into one of the falcon tubes and the electric wire was passed through the holes on the lids to the exterior. The lids of tubes were covered aluminium foil and autoclaved at 121^0C for 15 minutes. 30 mL of sterile nutrient broth was aseptically transferred into each of the sterile falcon tubes. A loopful of a pure colony was transferred into one of the falcon tubes and the tubes were placed on a rack. The inoculated medium and the negative control were degassed for at least 2 minutes using nitrogen gas and were incubated at 28^0C and 70 rpm for 20 days. After 20 days of incubation, each electrode was carefully transferred into an already degassed 250 mg/L of benzene in MSM. The peak current and potential of the negative control and each of the biofilms formed on the electrode by the isolate was determined at a scan rate of 10 mV/s using a potentiostat. While the incubated electrodes with biofilm served as the working electrodes, a platinum electrode was the reference electrode and a carbon clothe electrode with same measurement as the working electrode (2 cm X 3 cm) was the counter electrode. After the measurement, the percentage of each isolate's peak current above or below the peak current of negative control was ascertained by this equation,

$$\frac{\text{Peak current of isolates} - \text{Peak current of negative control}}{\text{peak current of negative control}} \times 100$$

Using the above formular, isolates that their electrochemical activities were at least 100% higher than the negative control were selected. While most of the isolates had good peak current greater than the negative control, the benchmark of 100% or more peak current greater than that of the negative control was to ensure that the best performers were

selected for the microcosm formulation for each matrix. Isolates 14, 11 and 6 from soil, sediment and groundwater respectively had a peak current within the benchmark that were considerably good numbers for microcosm formulation. The above method was repeated to test the electrochemical activity of the isolates against a positive control *Shewanella oneidensis* as a known electro active bacterium.

2.3.6 Assessment of biosurfactant production by the pure colonies isolated from petroleum hydrocarbon polluted matrices.

48 hours prior to experimental set up, each of the pure colonies was sub-cultured on a nutrient agar plate. Carbon cloth electrodes measuring 2 cm X 3 cm was cut and an electrical wire measuring approximately 20 cm soldered to it. A small hole was bore at the centre of 45 ml falcon tubes lids. Each of the soldered electrodes was transferred into one of the falcon tubes and the electric wire was passed through the holes on the lids to the exterior. The lids of tubes were covered with aluminium foil and autoclave at 121^0C for 15 minutes. 30 mL of sterile nutrient broth was aseptically transferred into each of the sterile falcon tubes. A loopful of a pure colony was transferred into one of the falcon tubes and the tubes were placed on a rack. The inoculated medium and the negative control were degassed for at least 2 minutes using nitrogen gas and were incubated at 28^0C and 70 rpm for 20 days. After 20 days of incubation, 4 mL of the solution was centrifuged at 10,000 rpm for 5 minutes. 2 mL of the supernatant believed to contain the biosurfactant was collected into a fresh tube and a 2 mL of vegetable oil was added to each of the isolates' supernatant. Each tube was vortexed for 2 minutes and incubated at 28^0C for 48 hours. The biosurfactant emulsified layer of the oil was measured, and the Emulsification Index (EI24) was determined as previously carried out by (Cooper and Goldenberg, 1987; Datta et al., 2018) using this equation

Emulsification index (EI24) = Height of the emulsified layer X 100

Total height of oil

2.4 Metagenomics analysis of petroleum hydrocarbon polluted environmental samples (groundwater, soil and sediment)

DNA extraction for each of the petroleum hydrocarbon polluted matrices (groundwater, soil and sediment) was carried out using Sigma Alrich GenElute Soil DNA isolation kit. The procedure was carried out according to the manufacturer's instructions. Nanodrop was used to measure both the concentration and purity of the extracted DNA to ensure that they met the specification required by Novegene. The DNA samples were shipped in dry ice to Novogene for metagenomics analysis. The region of analysis used by Novogene was 16S (V3-V4) and Illumina Sequencing PE250 was the Sequencing Platform & Strategy.

2.5 Microbial Fuel Cells (MFCs) outputs for pure strains and microcosms isolated from the polluted petroleum hydrocarbon matrices

This section was aimed at assessing different MCFs outputs of a good performing pure strain compared to all the strains from each matrix being put together to form a microcosm for each matrix. The microorganisms were those that were screened and selected from section 2.3 above. The comparison between the pure strains and the microcosm experiment was to ascertain which of the inocula was suitable for the MFCs experiments going forward.

2.5.1 Preparation of MFCs chambers

Eight pairs of MFCs H-type chambers were washed and allowed to dry. A rubber ring was attached to each of the chambers' ring ports. A Proton Exchange Membrane (PEM) was cut to size and was attached in the rubber rings and a clamp was used to hold two chambers

together. To ensure that the set up was properly aligned, it was tested for leaks by filling both chambers to the brim with water and was left over night. After confirmation of no leaks, the water was carefully discarded and allowed to dry at room temperature. The chambers were with their lids and the lids were covered with aluminium foil to reduce the chances of any contaminant getting into the chambers. This was then autoclaved at 121^{O}C for 15 minutes.

2.5.2 Inoculum preparation

One of the pure strains from each of the polluted matrix (soil. Sediment and groundwater) with significant metal clearance (the brownish-red colour of iron III oxide and the black colour of manganese IV oxide becoming less dense and transparent when compared with the negative control in section **2.3.2.4** were selected. Half of the content (10 ml) of each bottle was aseptically withdrawn and replaced with fresh sterile 8 ml mix of MSM, vitamin and trace elements mix as prepared in section **2.3.1.1**, 1ml of a benzene stock and Iron III oxide stock as prepared in sections **2.3.1.2** and **2.3.1.3** respectively. This was incubated at 28^{O}C and 150rpm for 72hrs. Subsequently, all the colonies from each matrix with the ability to degrade petroleum hydrocarbon, significant electrochemical activity and although not mandatory is able to produce biosurfactant/bioemulsifier were selected for a microcosm set up. Details of the selection criteria are in 2.3 above. The total number of pure colonies that exhibited at least 2 of the selection criteria (ability to degrade petroleum hydrocarbon and good electrochemical activity) are as follows: soil with 14 isolates, sediment 11 while groundwater had 6. An MFC experiment was set up separately for the good performing pure strains and the microcosm for each of the matrix.

2.5.3 MFCs Connection, inoculation and incubation

Solid phase electrodes made of carbon fibre electrode material from University of Reading was cut into 25 cm^2 surface area. The electrodes were connected to an electrical wire by

soldering with copper wire. The electrodes were immersed with 70% ethanol for 24 hours so as to sterilise them. Prior to use, the electrodes were rinsed with sterile deionised water and was transferred into both chambers and the soldered electrical wires were inserted through a small hole in the lid to the exterior of the chambers. 200 ml of 0.1 M of potassium ferricyanide dissolved in 0.1 M phosphate buffer solution was transferred into each of the cathode chambers and the lids were loosely covered to allow the flow of air into the chambers. For the anode chambers, 160 ml of sterile MSM, vitamin and trace elements mix as prepared in section **2.3.1.1** was aseptically measured and transferred into each of the anode chamber. This was followed by the addition of the 20 ml of a benzene stock as prepared in section **2.3.1.2.** The 20 ml inoculum prepared above was then transferred into the anode chamber. This was then degassed with nitrogen gas and the lids tightly covered and sealed with parafilm to prevent the flow of air into the chambers. The anode and the cathode chambers were then connected with a resistor (2,200 Ohms). The MFCs were then connected to a data logger (Picolog ADC-24, Pico Technology, UK). Real-time voltage outputs were transmitted and recorded at interval of 10 mins throughout the experiment on the picolog software in a computer. This same experiment was repeated using the microcosm from each of the matrix. The various outputs for both the microcosm and the pure colony experiments are presented in **Chapter 4.**

2.5.4 Evaluation of microbial fuel cell performance

2.5.4.1 Electrochemical analysis

The assessment of MFCs performance for all the studies was based on voltage and current outputs. Electric current (I) flowing through the external load was estimated through the use of employed resistance (Ω) and measured potentials (E). Each of the current flowing through the external load of the MFC and the power generated are determined by Ohms law. The

power density curve method is used to obtain the maximum power density (Logan, 2008). Power density *P* (mWm^{-2}) is calculated using the equation

$$\mathrm{P} = \frac{I \times E}{A}$$

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}) was normalized to the projected total surface area of the anode.

2.5.4.2 Polarisation testing.

This test was carried out by temporary replacement of the Resistor with a Decade Box

A polarisation curve (voltage vs current density) was generated using the external resistance method. This assisted to determine the microbial fuel cells' internal resistance and other resistances to electron connection.

Method:

1. The MFC's resistor was removed and replaced with a decade box.
2. To remove all resistance, no switch was switched on until the voltage for the given MFC stabilises to get the Open Circuit Voltage.
3. Different resistance values ranging from 4 Ω to 500,000 Ω and the voltage for each MFCs was recorded. As it would take too long to wait for all to stabilise, set a timer for 10 minutes for each value resistor to stabilise and record voltage at that moment for each.
4. When the collection of the data was completed, the decade box was replaced with the resistor and the MFCs were reconnected to the picolog channels for continued data

recording. The current and power densities were derived using the above formular on Microsoft Excel.

2.6 Microbial Fuel Cell integrated with microcosm formulated from polluted petroleum hydrocarbon samples (soil, sediment and groundwater).

The MFC configuration was based on the matrix type and was integrated with microcosm that were isolated from that specific matrix. Microcosm formulated from each pf the matrix was used solely in a laboratory experiment that closely mimics the petroleum polluted matrix that the microorganisms were isolated from as recorded in Chapter 3. The three microcosms formulated from the petroleum polluted soil, sediment and groundwater were used in experiments that closely mimic soil, sediment and groundwater respectively. Therefore, all subsequent experiments were independent of one another and were significantly different from the microcosm used, matrix design, MFCs configuration, bioreactors and other variable of interest.

2.6.1 Criteria for pure strains selection for microcosms formulation

The same criteria were used for the selection of pure strains across the different matrices (soil, sediment and groundwater). Each microorganism isolated in section 3.2 and section 3.3 was subjected to anaerobic conditions by inoculating each on Mueller Hinton Agar plate that were placed in anaerobic jars and anaerobic generating sachet (Oxoid™ AnaeroGen™ 2.5L Sachet) from Thermofisher Scientific was put into each jar and the lid of the jar was immediately closed. Each was also incubated under aerobic conditions by incubating plates containing each strain directly into the incubator. The aerobic and anaerobic growth conditions were to ensure that each pure strain that will form the microcosm for each matrix was a facultative anaerobe (having the ability to grow in both aerobic and anaerobic

conditions). Each facultative aerobe isolated was genomically identified using 16s rRNA and those that were identified to be either an emerging or pathogenic microbes were destroyed by autoclaving at 121^0 C for 45 minutes. Those whose gene did not relate with any pathogen results in Table 3.1, Table 3.2, Table 3.3, Table 3.4, Table 3.5 and Table 3.6 were subjected to the next level of screening. Electrochemical activity of each of the isolates and a negative control was ascertained by the peak current generated by each isolate and the negative control. From the results in Table 3.7, Table 3.8, Table 3.9. Table 3.10, Table 3.11 and Table 3.12, isolate whose peak current was greater than that of the negative control by 100% or more were selected to be among the strains to form the microcosm. Each isolate in Table 3.1, Table 3.2, Table 3.3, Table 3.4, Table 3.5 and Table 3.6 was still subjected to a biosurfactant production screening. Isolates that produced biosurfactant in Figure 3.16, Figure 3.17 and Figure 3.18 were among the microcosm formulation. Following the screening process above, 14, 11 and 6 pure strains were selected for soil, sediment and groundwater respectively for the microcosm formulation for each matrix.

2.6.2 MFC's inoculant (microcosm)

A loopful of each of the selected pure strains in section 3.8.1 was inoculated into 20 ml of nutrient broth and incubated at 28^0C for 24 hrs. These were stored in the refrigerator and used as stock culture. Prior to MFC setup, 10 ml of each stock was transferred into 90 ml of nutrient broth in a 200 ml conical flask and incubated in a sharker at 100RPM and 28^0C for 24 hrs. To formulate the microcosm for each matrix, the incubated pure strains from each matrix are mixed in a 2000 ml conical flask using a magnetic stirrer. Each of the microcosm was measured to be 10% of the total components of the bioreactor. For instance, 20 ml of the mixed 6 groundwater pure strains (microcosm) was added to the MFC bioreactor that already contained 180 ml (20 ml pollutant stock and 160 ml of water).

2.6.3 Types of MFCs bioreactors and materials used for the different matrices

The different contaminated matrices (soil sediment or groundwater) require different technological interventions in terms of reactors' configuration that can be used for either in-situ or ex-situ bioelectrochemical remediation of the petroleum hydrocarbon pollutants. Figures 2.2-2.4 show the reactors that were used in the microcosm studies for the different matrices. Electrodes that can withstand corrosion were prioritised for use. A cation exchange membrane (CEM, Membranes International, USA) was used to separate the anode chambers from the cathode chambers and an external resistance of 2000 Ohms was employed on each BES. Electrodes were fabricated based on the type of matrix**.**

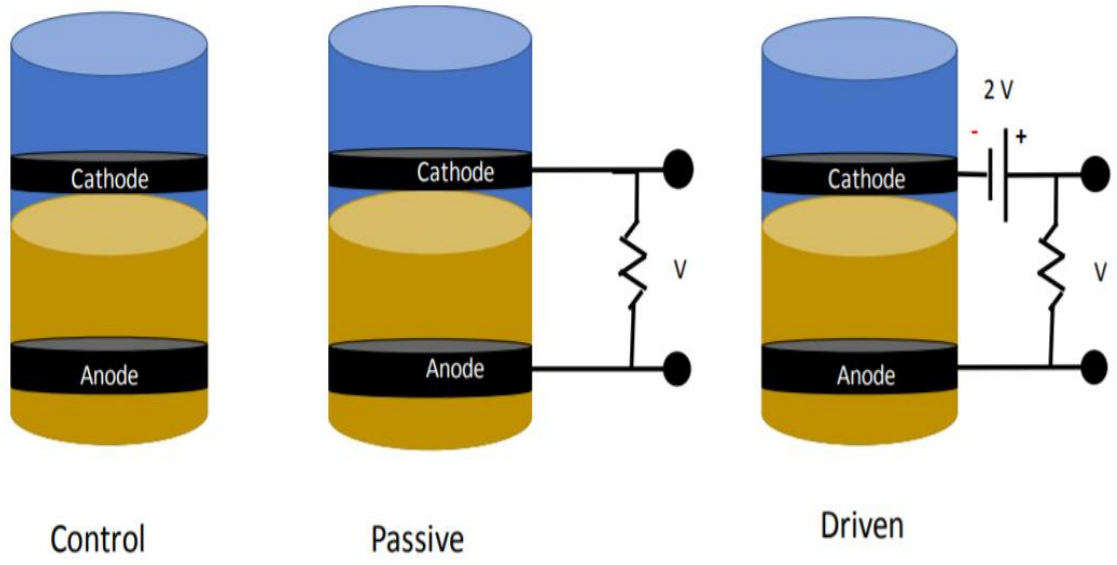
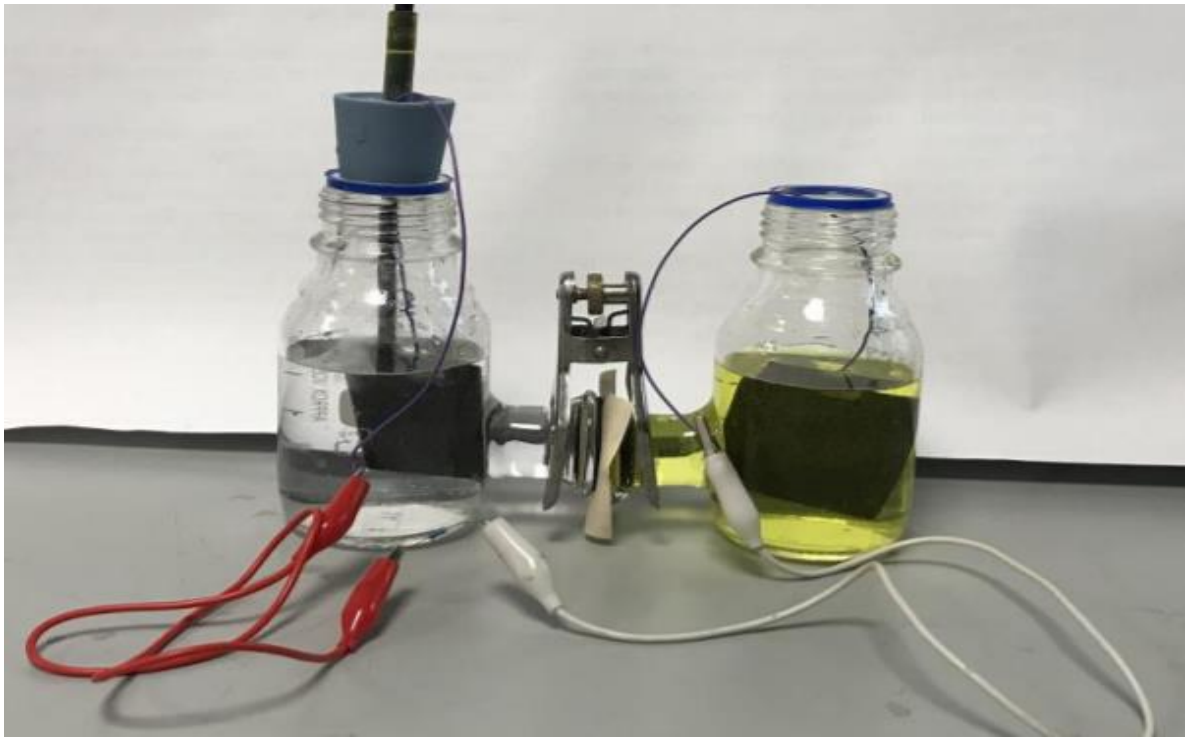


Figure 2.2 Microbial fuel cell configuration used for the investigation of petroleum hydrocarbon remediation in contaminated sediment.



A

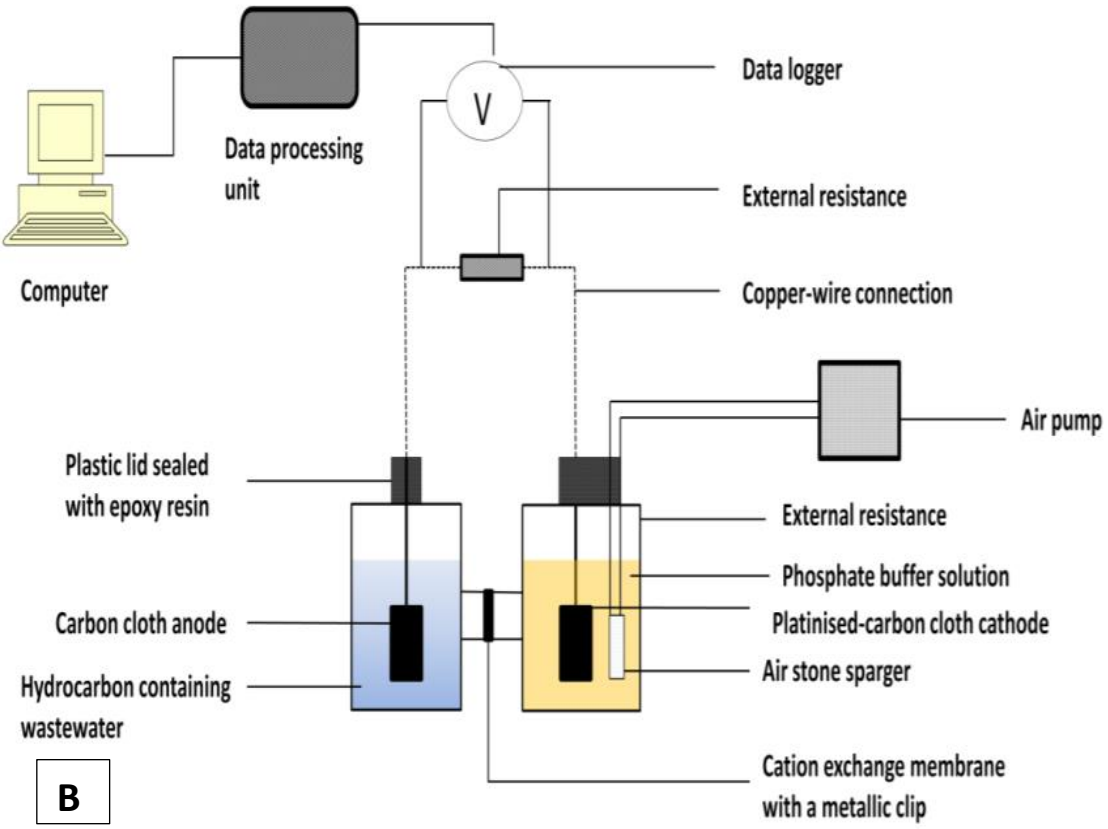


Figure 2.3 A and B are photo and schematic of an H-type microbial fuel cell configuration for the investigation of petroleum hydrocarbon remediation in contaminated groundwater (Adelaja, 2015).

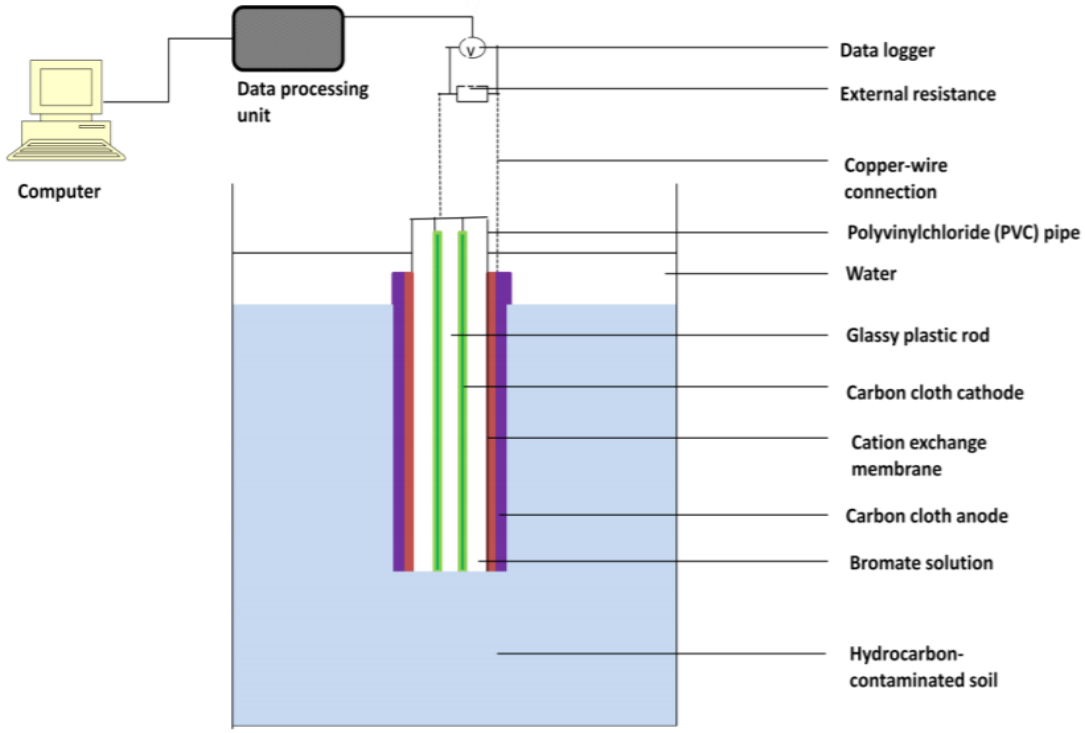


Figure 2.4 Schematic of a microbial fuel cell configuration used for the bioelectrochemical remediation of petroleum hydrocarbons in soil (Adelaja, 2015).

2.6.4 Comparing the effects of chemical surfactants and biosurfactants on phenanthrene biodegradation and degradation in Microbial fuel cell

This method was designed to test the impacts of supplementing the groundwater isolates (microcosm) with chemical surfactant and biosurfactants in phenanthrene degradation with a negative control MFC that only contained the pollutants and had neither the microcosm nor surfactant added. It is important to state that at the screening state 1 out of the 6 groundwater isolates as recorded in **Chapter 3 Figure 3.17** had emulsified layer with oil which indicated some level of biosurfactant production but not as much as the layered of the sediment isolates. The biosurfactant used was collected from a sediment isolate that had the best emulsified layer in **Chapter 3 Figure 3.16**. The isolate was inoculated into nutrient broth and incubated at 28^0C and 100RPM for 48 hours. This was centrifuge at 3500RPM for 30 minutes and the supernatant was collected. 250 mg/L of chemical surfactant

(polyoxyethylene sorbitan monooleate also known as Tween 80) the same chemical surfactant that was used by Adelaja, 2015. The experiment was carried out in a H-type MFC divided into 4 different categories of experimental set up. 1. 10% Tween 80 supplement 2. 10% biosurfactant supplement 3. The microbe alone 4. A negative control without microbes and without surfactant supplement. The total working volume was 200 ml in a 250 ml bioreactor capacity. All contained 200 mg/L of benzene, 100 mg/L of phenanthrene and MSM that contained trace element and trace vitamins details of these is 2.3.1.1above. The various set ups were constituted as follows

Tween 80:

160 ml of MSM + trace elements + trace vitamins, 20 ml of 2.5 g/L Tween 80 stock, 20 ml of groundwater microcosm and 228 ul mix of 2g and 1 g of benzene and phenanthrene stock to attain the 200 mg/L and 100 mg/L of benzene and phenanthrene target for the final bioreactor concentration.

Biosurfactant:

160 ml of MSM + trace elements + trace vitamins, 20 ml of biosurfactant, 20 ml of groundwater microcosm and 228 ul mix of 2 g and 1 g of benzene and phenanthrene stock to attain the 200 mg/L and 100 mg/L of benzene and phenanthrene target for the final bioreactor concentration. The biosurfactant used was produced a microorganism that produced the biosurfactant with the highest oil emulsified layer labelled (A) in Figure 3.16 isolated from the PH polluted sediment. Prior to the experiment, 10 ml of the isolate stock was inoculated into 90 ml of nutrient broth in a 200 ml conical flask and was incubated in a shaker at 100RPM and 28^0C for 48 hrs. After the 48 hrs, it was transferred into tubes and

centrifuged at 4500RPM for 30 minutes. The supernatant was used for the biosurfactant supplement.

Groundwater microcosm alone:

160 ml of MSM + trace elements + trace vitamins, 20 ml of water, 20 ml of groundwater microcosm and 228 ul mix of 2 g and 1 g of benzene and phenanthrene stock to attain the 200 mg/L and 100 mg/L of benzene and phenanthrene target for the final bioreactor concentration.

Negative control:

160 ml of MSM + trace elements + trace vitamins, 40 ml of water and 228 ul mix of 2g and 1 g of benzene and phenanthrene stock respectively to attain the 200 mg/L and 100 mg/L of benzene and phenanthrene target for the final bioreactor concentration.

All the above set ups (Tween 80, biosurfactant and negative control) were degassed using nitrogen gas, incubated at 28^0C and were connected to picolog channels and the voltages were recorded for 19 days.

The reading of the polarisation curve was carried out as already described above. The samples were prepared for HPLC analysis by mixing 1 ml of each sample with 1 ml of methanol and vortexed for 2 minutes. This was then centrifuge at 12000RPM for 4 minutes and the supernatant was collected in vials and analysed using the same HPLC as above. The HPLC analysis was carried out at the beginning and at the end of the experiment.

2.6.2 Effects of biochar on the degradation of petroleum hydrocarbon and its impacts on electrode's Radius Of Influence (ROI) in soil microbial fuel cell.

Materials

The materials below were used to carry out the experiment:

Water, microcosm Boughton kaloam loam cricket dressing soil containing 25-28% clay content was purchased from Boughton,

618g UD carbon fibre cloth 50 k-600 mm wide electrode purchased from East coast fibreglass supplies,

Carbon fibre electrode tissue 10 cm x 2 cm from University of Reading,

Electrical wire, soldering wire, benzene, phenanthrene, Potassium ferricyanide solution as already prepared above, semi-permeable membrane, 200 ml cylinder and a driller, gorilla glue, plastic buckets.

Method

To create an electrode for the soil MFC, several holes were drilled on 200 ml plastic cylinder within the region of the 0-200 ml calibration. The UD carbon fibre cloth 50k-600mm wide electrode was cut into 17 cm x 19 cm for width and length respectively and the semi-permeable was also cut to the same size with little of 2cm allowance for overlap. The Carbon fibre electrode tissue 10cm x 2cm was cut into 2 cm X 15 cm for width and length respectively. An electric wire was then attached to the electrodes. The semi-permeable membrane was wrapped around the cylinder and was then fastened tight. To ensure that leaks were prevented, a gorilla glue was used to cover all the openings of the overlapped edges and more concentrated at the base of the cylinder. This were then kept on a tray and 200 ml of water was poured and left overnight to ensure that there were no leakages. The already cut 17 cm

x 19 cm UD carbon fibre cloth 50k-600mm electrode was wrapped and tighten unto the cylinder.

6 Kg of soil was weighed unto the separate buckets and another containing 5% (300 g) of activated charcoal added to 5.7 kg of soil.

To ascertain the effects of biochar on electrode radius of influence the experiment was designed into 3 different categories as follows:

1. Soil sample mixed with 5% biochar
2. Soil sample without biochar
3. Negative control to mimic natural attenuation

Prior to the experiment, each of the soil isolates were separately inoculated into nutrient broth and incubated at 28^0C and 100RPM for 24 hours. These isolates were mixed before the whole bioreactors contents was put together. 200 ml of potassium ferricyanide as prepared above was poured into the 200 ml cylinder already wrapped with the semi-permeable membrane and the electrode. The already soldered Carbon fibre electrode tissue 2 cm x 15 cm ferricyanide solution to serve as the cathode. This was then placed in the buckets for the 1st and 2nd set up. The total target volume for each bioreactor was 8400 g (soil and liquid mixture) regardless of either it contained biochar or not, the already measured 6000 g of soil was mix with 16.8 g and 8.4 g of benzene and phenanthrene respectively. This soil was gradually transferred into the buckets with the electrode placed at the centre for the 1st and 2nd set up and others without electrode for the 3rd set up. 2400 ml of water that contained 840 ml of the microcosm was gradually transferred to the 1st and 2nd set up above while 2400 ml of water only without microcosms was transferred to the 3rd set up. All these were carried out in the fume cabinet. The anode in 1st and 2nd set up were connected to their corresponding cathode with a resistor (2,200 Ohms) and all the three set ups were then

incubated at 28^0C. It is important to note that the benzene and phenanthrene mixed with the soil was calculated to give an overall concentration of 200 mg/L and 100 mg/L of benzene and phenanthrene in bioreactor respectively. The 1st and 2nd MFC set ups were then connected to a data logger (Picolog ADC-24, Pico Technology, UK). Real-time voltage outputs were transmitted and recorded at interval of 10 mins throughout the experiment on the picolog software in a computer. The various MFC outputs were taken and recorded and the evaluation of the MFCs were carried out as described earlier 2.5.4.

To ascertain the effects of biochar on the radius of influence of the electrode, 2 g of soil each was collected at 4 cm and 8 cm distance from the electrode from the 1st and 2nd set up while the negative control that had no electrode in it had 2 g also collected at different points and mixed. Each of these 2 g was mixed with 2 ml of methanol. Each of the sample was vortex for 2 minutes and was centrifuge at 12000RPM for 4 minutes and the supernatant was collected in vials and analysed using the same HPLC as above. The HPLC analysis was carried out at the beginning and at the end of the experiment.

2.6.3 Comparison between air breathing MFC and snorkel MFC configuration in the degradation of petroleum hydrocarbons in a mimic sediment microbial fuel cell

Materials

The materials used were as follow:

Water, microcosm

Boughton kaloam loam cricket dressing soil containing 25-28% of clay content purchased from Boughton

618g UD carbon fibre cloth 50 k-600 mm wide electrode purchased from East coast fibreglass supplies

Electrical wire, soldering wire, benzene, phenanthrene, Potassium ferricyanide solution as already prepared above, 100 ml cylinder, 2000 ml beakers, platinum black, activated charcoal, nafion solution.

Method

Preparation of electrodes

For snorkel, the UD carbon fibre cloth 50 k-600 mm wide electrode was cut into 14 cm x 12 cm for length and width respectively. 47 mg of platinum black and 423 mg of activated carbon were weighed and transferred into a 50 ml beaker. Nafion solution was then added to this and mixed together to form a paste. The already cut electrode was folded into equal halves (7 cm x 6 cm) and the paste was rubbed on one half of the electrode. This was repeated for the second electrode and was allowed to dry for 24 hours. After the electrodes dried, they were then wrapped around the 100 ml cylinders with the half coated with the paste placed at the bottom to serve as the anode while the second half without the paste was at the top to serve as the cathode.

Air breathing electrode made by cutting the UD carbon fibre cloth 50k-600mm wide electrode was cut into 14 cm x 12 cm into two equal halves (7 cm x 6 cm) and an electrical wire measuring about 20 cm long was firmly tied to the electrodes that served as the cathode and anode. This was duplicated.

To ascertain the effects of biosurfactant on degradation of phenanthrene the experiment was designed into 3 different categories as follows:

1. Snorkel electrode

2. Air breathing electrode
3. Negative control that only had the pollutants with neither electrode nor the microcosm

Prior to the experiment setup, each of the 14 soil isolates were separately inoculated unto nutrient broth and incubated at 28^0C and 100RPM for 24 hours. These isolates were mixed before the whole bioreactors contents was put together to form a microcosm. 1.5 g of soil was weighed and mixed with 6.4 g of benzene and 3.2 g of phenanthrene. Each of the snorkel electrode was placed at the centre of 2000 ml beakers and the soil mixed with benzene and phenanthrene was gradually transferred unto the beakers. For air breathing electrode set up, 750 g (half of the total quantity of the soil for the ioreactor) of the soil mixed with the pollutants was transferred unto the 2000 ml beaker after which the electrode that served as the anode was carefully placed the remaining half of the soil was transferred to cover it. For the negative control, the 1.5 g soil mixed with the pollutants was carefully transferred into 2000 ml beaker. 320 ml of the microcosm was mixed with 1380 ml of water was transferred into the 1^{st} and 2^{nd} set up. 1700 ml of water was transferred into the negative control. The negative control had neither microcosm nor electrons in them. It is important to note that the benzene and phenanthrene mixed with the soil was calculated to give an overall concentration of 200 mg/L and 100 mg/L of benzene and phenanthrene respectively in the bioreactor. All these were carried out in the fume cabinet. The air breathing electrode set up had their anode connected to their corresponding cathode with a resistor (2,200 Ohms). All the three setups were then incubated at 28^0C for 16 days. the air breathing electrode were then connected to a data logger (Picolog ADC-24, Pico Technology, UK). Real-time voltage outputs were transmitted and recorded at interval of 10 mins throughout the experiment on

the picolog software in a computer. The various MFC outputs were taken and recorded and the evaluation of the MFCs were carried out as described earlier.

2 g of soil from each of the samples collected and mixed with 2 ml of methanol. 1 ml of the supernatant from each of the setups was also collected and mixed with 1 ml of methanol. Each of the sample was vortex for 2 minutes. This was then centrifuge at 12000RPM for 4 minutes and the supernatant was collected in vials and analysed using the same HPLC as above. The HPLC analysis was carried out at the beginning and at the end of the experiment. This was done at the beginning and at the end of the experiment.

The experiments in this chapter were carried out in duplicates and repeated at least twice and their respective results are the average of the data for each experiment.

2.7 Experimental design

Table 2. 4 Rationale for the investigations that were carried out in this project.

Challenge	Hypothesis
Need for hydrocarbon degraders that are facultative anaerobes, EAB and could also produce biosurfactant that can be formulated into microcosms that could enhance biodegradation of petroleum hydrocarbon using BES	Petroleum hydrocarbon degraders could be isolated from petroleum hydrocarbon contaminated matrices (soil, sediment and groundwater). Using different culture-based techniques could promote the growth of different microorganisms that possess the desired characteristics that could enhance the degradation of petroleum hydrocarbon when integrated with BES Several strains have over the years been shown to degrade petroleum hydrocarbons, recently reviewed by [Xu et al., 2018]. To develop microbial consortia that could be used for bioaugmentation. These microbes would be screened for their potential for petroleum hydrocarbon degradation, electrochemical activity and biosurfactant production. The best strains are to be constituted together to form microcosms. Screening experiments will be carried out targeting the most efficient microorganisms that could enhance the degradation of PH pollutants.
Need to enhance the degradation of petroleum hydrocarbon in different MFCs set ups	Supplements such as biosurfactants and biochar when integrated to BES could enhance the biodegradation of petroleum hydrocarbon pollutants.
Technological challenge (identifying suitable MFC designs) of carrying out electro-based reactions in unconventional matrices e.g. sediments	Investigate different designs of MFC in bioreactors that could be deployed for in-situ/ ex-situ remediation of petroleum hydrocarbons could help in identifying the most suitable for a sediment matrix.

Chapter 3: Prospecting for Petroleum hydrocarbon degraders, biosurfactant producers and electrochemically-active bacteria

Overview

Petroleum hydrocarbons are one of the toxic and recalcitrant compounds that are often found as pollutants in our environment. Due to their negative impacts on the ecosystem and human health, these compounds have been considered a priority for remediation (UNEP, 2011; Sajna et al., 2015; Varjani, 2017). Hence several remediation approaches including physical, biological and chemical methods have been employed with the aim of enhancing their degradation (Lim et al. 2016). As well intended as most of these methods may be, some are ineffective, expensive, not environmentally friend and not safe. The challenges of existing remediation approaches have led to several efforts to either design new technology or improve on existing ones that could overcome these challenges. Bioelectrochemical systems (BES) an emerging technology have in recent time been considered a viable option for petroleum hydrocarbons remediation due to its several advantages that include: 1. Electrodes use in an optimal BES are able to provide an unlimited number of electron receptors and donors that facilitate microbial redox reactions that enhances the degradation of pollutants thereby, shortening the remediation time (Wang et al., 2020a) and 2. help to prevent the use of chemicals (reductants and oxidants) that can cause secondary pollution (Beretta et al., 2019). 3. Compared to other methods the cost of installation and maintenance of BES is cheaper. 4. It is suitable for the remediation of a wide range of pollutants because of its ability to provide both reduction and oxidation transformation (Wu et al., 2018). 5. Its ability to simultaneously degrade pollutants and generate electricity makes the process of remediation either energy positive or neutral. 6. The progress of the degradation can be monitored remotely. These advantages can be attributed to the technology's ability to use a combination of both electrochemical and biological processes that utilize electroactive microorganisms. These electroactive microorganisms serve as catalysts for the reduction or

oxidation reactions of inorganic or organic electron donors and transfer of electrons to a solid-state electrode. BES is divided into Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs). While MFCs is an entirely spontaneous reaction of substrate degradation and the production of electricity, MECs requires a small energy input to drive the entire process of pollutant degradation and simultaneously produce hydrogen/methane/water (Cario et al., 2019). Several petroleum hydrocarbons remediation studies have been carried out using MFCs with substantial success (Adelaja et al., 2015, Wang et al. 2012, Morris and Jin 2012). However, just like any other promising technology, BES has its own limitations and there have been constant efforts towards addressing them to optimise the system. One of the notable key limitations of BES is the knowledge and understanding of the microorganisms involve in the remediation process. Wang et al., 2020b while acknowledging the potentials of BES, suggested that the first hurdle is to overcome the lack of knowledge on electroactive microorganisms and their contribution to the remediation process. Having a means for cultivating these microorganisms so that the isolates can be integrated at the beginning of a remediation process. Having the knowledge of the key roles that indigenous microorganisms play in bioremediation, we commenced a study to prospect for electrochemically-active and hydrocarbon-degrading microorganisms. The samples for the study were collected from prolonged petroleum hydrocarbon polluted matrices (soil, sediment and groundwater) in the Niger-Delta region of Nigeria. This chapter report the result of physicochemical properties of the polluted petroleum hydrocarbon samples from environmental matrices (groundwater, soil and sediment) that were used for isolating the microorganisms of interest, metagenomic analysis of microbial community of each matrix, isolated microorganisms using the two culture-based techniques, electrochemical and biosurfactant production of each isolate. This

gives an overall insight on the selection criteria of isolates that made up the formulated microbial consortia that were integrated into BES.

3.1 Characterization of contaminated samples

The result of the physicochemical analysis of the samples from environmental matrices (groundwater, soil and sediment) polluted with petroleum hydrocarbons. that was carried out by i2 Analytical Ltd, 7 Woodshots Meadow, Croxley Green Business Park Watford, United Kingdom is in **Appendix 1.** The physicochemical characteristics of the sites is important in understanding bioremediation parameters such as nature of contaminants, environmental conditions like pH, presence/absence of electron acceptors etc.

3.2 General medium method to enrichment medium method

The results below are those that were isolated from the general-purpose medium and thereafter, subjected to the screening pressures (benzene as the sole carbon and manganese IV oxide and iron III oxide as electron acceptor) in Method (section 2.3.2).

3.2.1 Microbial colonies isolated based on the General medium method to enrichment medium

Twenty-three bacterial colonies were isolated from the three matrices grown on Mueller Hinton agar maintained under aerobic conditions at 28^0C. The matrices with the highest number of distinctive colonies were soil and sediment having 10 colonies each while groundwater had the least with three colonies. Although the technology (BES) is operated under anaerobic conditions facultative anaerobes such as *Pseudomonas spp*. have been reported to be utilised in the cathodic and anodic chambers of BES during hydrocarbon degradation (Clauwaert et al. 2007; Erable et al. 2010, Adelaja et al., 2015). Therefore, it was considered that inoculated sample should be grown under aerobic conditions so as to give

facultative anaerobes a competitive advantage over strict anaerobes. Out of the twenty-three pure colonies that grew under aerobic conditions only fourteen of them grew under anaerobic conditions when sub-cultured on MHA at 28^0C. Nine colonies out of the ten colonies isolated from soil under aerobic condition were able to grow under anaerobic conditions. This makes soil the highest matrix to have strains that were isolated from aerobic and anaerobic conditions the highest number of facultative anaerobes. While sediment had five out of its ten isolates that could grow under aerobic and anaerobic conditions, neither of the three colonies isolated from groundwater under aerobic condition could grow anaerobically. When the nine colonies from soil sample and five from the sediment sample that grew under aerobic and anaerobic condition were enriched with benzene and electron acceptor (Iron III oxide and Manganese IV oxide) only two colonies one each from soil and sediment showed a fair colour clearance of the metals for iron III oxide and manganese IV oxide respectively. Using NCBI megablast these isolates were identified to be highly similar to *Pseudomonas fluorescens strain KT3* and *Pseudomonas azotoformans* soil and sediment respectively. These species belong to the genus Pseudomonas that are facultative anaerobes and some of its species have been reported to degrade petroleum hydrocarbons (Adelaja et al., 2014., Friman et al., 2012).

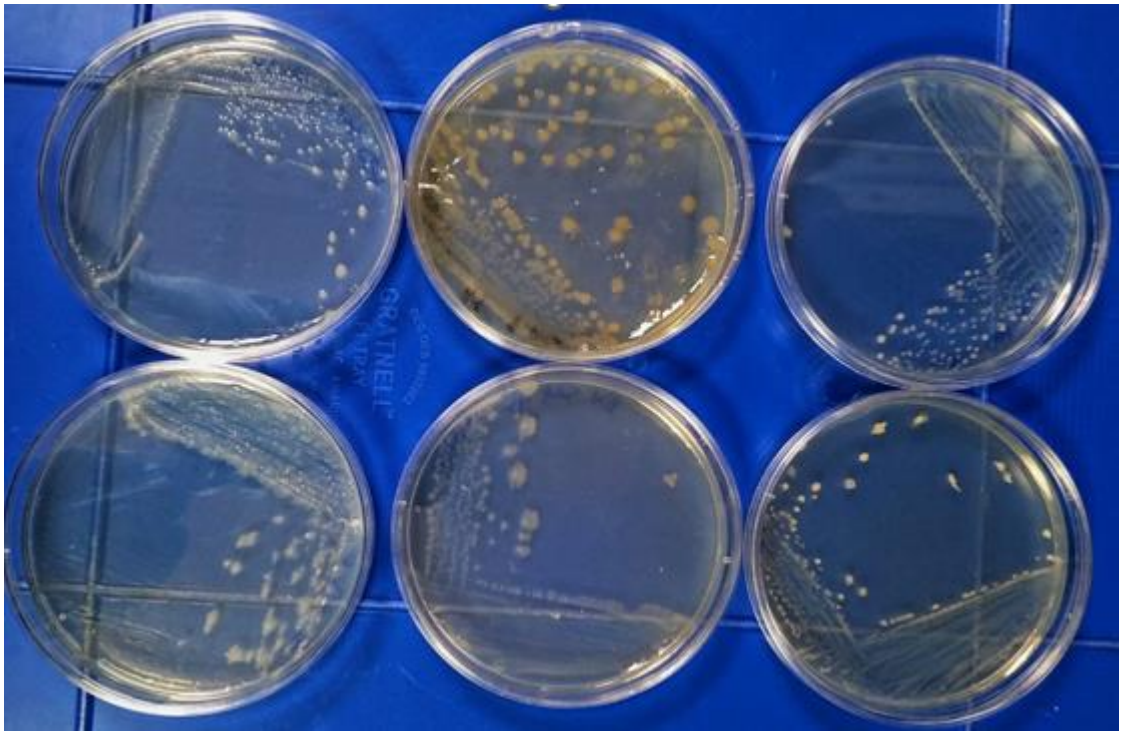


Figure 3.1 Pure colonies isolated from polluted petroleum hydrocarbon matrices (soil, sediment and groundwater) grown on Mueller Hinton agar at 28^{0}C under anaerobic condition.

3.2.2 Colour clearance of electron acceptor by pure colonies isolated using the general medium method to enrichment medium method.

The samples inoculated on MHA and incubated under anaerobic condition at 28^{0}C had fifteen, seven and five pure colonies for soil, sediment and groundwater respectively. When these pure colonies were enriched with benzene and the electron acceptor (iron III oxide and manganese IV oxide), there was medium to high colour clearance of the metals by almost all the pure colonies. Ten, six and five colonies for soil, sediment and groundwater respectively showed significant clearance of the metals while others did not have any impact on the metals' colour. The colour clearance of the pure strains is presented in **Figure 3.2.** Having the pure strains to be closely related to the genus Pseudomonas as the dominant species in soil and groundwater in **Table 3.1 and 3.2** is not a surprise as species from this genus have been previously recorded to be hydrocarbon degraders (Daghio, 2017). Furthermore, species from this genus have also been successfully utilised in MFCs set up for the degradation of

petroleum hydrocarbons (Adelaja et al., 2014., Friman et al., 2012). While three pure soil isolates (*Ewingella Americana cqsV12, Pseudomonas azotoformans strain P187, Enterobacter ludwigii strain MBPSL*) had fair to good colour clearance for both iron III oxide and manganese IV oxide, other pure isolates from groundwater and sediment had colour clearance for either iron III oxide or manganese IV oxide. The versatility of the three soil isolates in utilising both electron acceptors align with Myers and Nealson (1988) report that that certain microorganisms have the ability to utilise more than one compound as terminal electron acceptors.

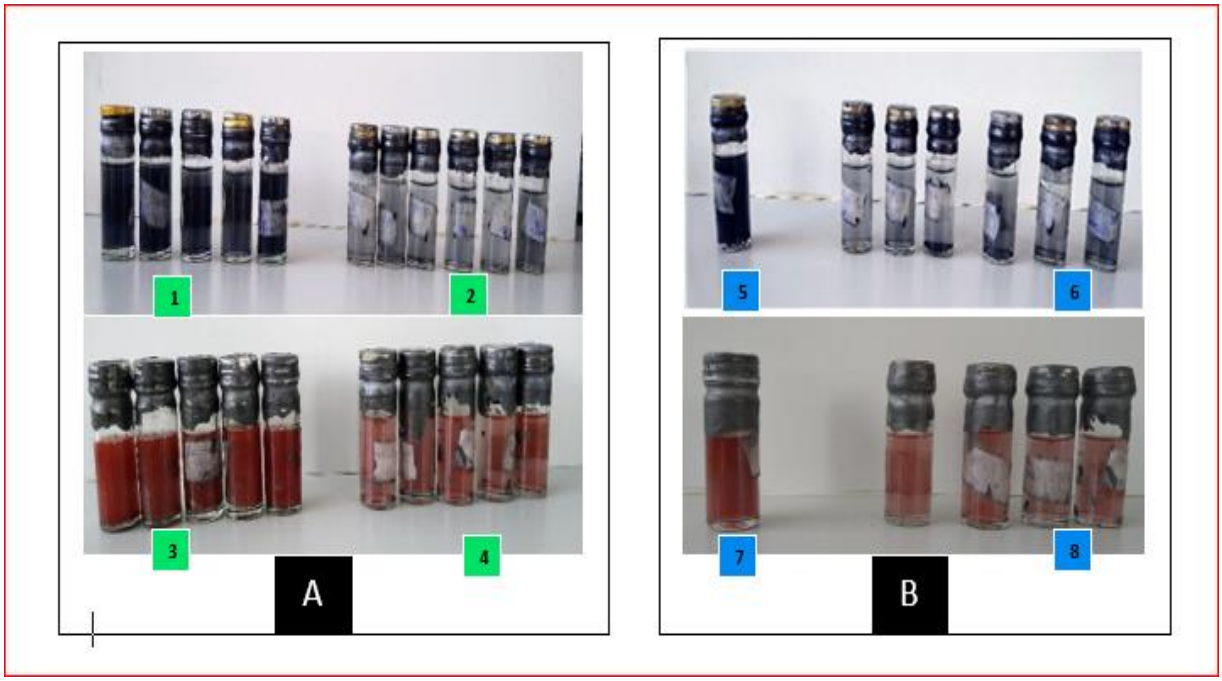


Figure 3.2 The bottles labelled 1 and 3 in picture A shows inoculated pure strain without significant colour clearance for manganese IV oxide and iron III oxide while 2 and 4 had significant clearance for manganese IV oxide and iron III oxide. Bottles labelled 5 and 7 in picture B are the negative control while 6 and 8 are inoculated single celled colony with significant clearance for manganese IV oxide and iron III oxide.

3.3 Enrichment medium method to general medium method

The results below are the environmental samples that were initially subjected to the screening pressures (benzene as the sole carbon and manganese IV oxide and iron III oxide as electron acceptor) and thereafter, isolated using a general medium in method 2.3.3.

3.3.1 Colour clearance of electron acceptors and isolation of microorganisms based on the enrichment medium method to general medium method.

After 7 days of incubation, the samples containing groundwater showed close to total colour (black and brownish red for Manganese IV oxide and iron III oxide respectively) clearance as presented **Figure 3.3**. This was an indication that the microbial community present in the sample had utilised the benzene, Iron III oxide and Manganese IV oxide. It took the microbial communities in groundwater a shorter time (7 days) to achieve better colour clearance than the pure colonies in **Figure 3.2.** This aligns with the believe that BES hydrocarbon remediation is better facilitated by consortia of microorganisms that work in synergy to breakdown recalcitrant hydrocarbons into different metabolites (Wang et. al., 2020a). However, this does not negate the fact that single celled pure colony can be electrochemically active and still degrade petroleum hydrocarbons. Although *Geobacter* an EAB has been recorded in the past to also degrade petroleum hydrocarbon, but this has been considered kinetically and energetically difficult for one single cell to degrade complex hydrocarbon and still conduct extracellular electron transfer (Lovley 2006). From the result on **Table 3.4-3.6** microorganisms identified to belong majorly to *Pseudomonas sp.* that are popularly known for their petroleum hydrocarbon degrading abilities have also been seen to utilised both manganese IV oxide and iron III oxide as terminal electron acceptors. Comparing the result of the enrichment environmental samples that contained consortia of microorganisms and those inoculated

with a single strain, the consortia were observed to have faster and better result within a shorter time than the enrichment media inoculated with the single strain microorganism in **Figure 3.2**. This gave a strong indication that going forward, microorganisms that are electrochemically active and have petroleum hydrocarbon degrading abilities when combined in a microcosm set up are most likely to give better result than pure strain inoculum.

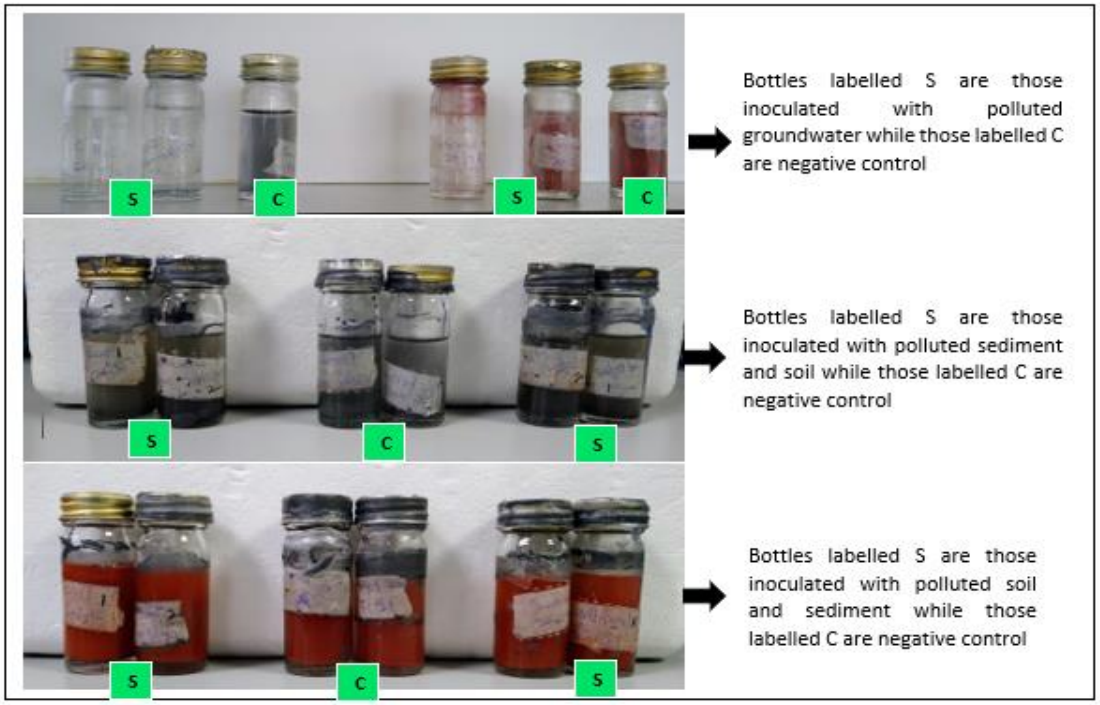


Figure 3. 3 The enrichment bottles inoculated with groundwater showing very good colour clearance for manganese IV oxide and iron III oxide to its control, while bottles inoculated with soil and sediment being turbid samples were difficult to ascertain the difference with their control.

3.3.2 High-performance liquid chromatography (HPLC) analysis of soil and sediment samples.

Due to the turbidity of the soil and sediment samples, ascertaining the level of the metal colour clearance between those inoculated with the environmental samples and the negative control was difficult. Hence, the benzene concentration analysis using HPLC was carried out and the result is presented in **Appendix 2** shows the expected retention time for benzene BES minimal defined medium components and the result for the environmental samples. The

result for the environmental samples showed no detectable peak for benzene while the respective negative control showed benzene peaks. This indicates the presence of petroleum hydrocarbon degrading consortia with electrochemical activity potential for both the polluted sediment and soil.

3.4 Identified microbial community and pure strains from the polluted petroleum polluted matrices

3.4.1 Overview

Metagenomics (MGs) is a technique that is used to directly analyse genetic composition of environmental samples. This form of analysis is culture independent and is useful in understanding an environmental remediation process that is driven either by microorganisms and/or their gene products (Wani et al., 2022). This approach is divided into sequence based and functional based metagenomics. The sequence-based metagenomics analyses microbial community as regards to the species composition and abundance in each sample. On the hand, functional based metagenomics is used in screening for new functional genes and to detect new bioactive substances (Zhang 2021). The insight provided by MGs analysis is considered useful in bioremediation by providing information that could help optimise the process that include but not limited to 1. understanding the efficient bioremediation mechanism pathways that are driven by microorganisms. 2. Using microbial diversity and specific genes to serve as pollution biomarkers 3. Providing information for efficient bioremediation strategies and models development. 4. Identifying and selecting efficient microorganisms for bioremediation (Wani et al., 2022) and understanding the interaction between microorganisms and environmental the details within a polluted matrix (Mishra et al., 2021). 5. Discovery of new bioactive substances that enhance the remediation process (Zhang 2021). As important as the role of MGs is to bioremediation, there is still the need to

combine MGs and culture-based techniques in other to fully understand the microorganisms that play key roles in the remediation process. Thereby, helping in the development of a more efficient bioremediation technique (Wani et al., 2022). In an attempt to optimize the bioremediation process, both the metagenomics and two culture-based approaches were employed. The metagenomics analysis carried out involved the direct DNA extraction of genomic materials from the petroleum hydrocarbon polluted environmental samples with the aim of ascertaining the species composition and abundance in each sample using New Generation Sequencing. On the other hand, the culture-based approaches employed used a selection pressures (anaerobic growth condition, petroleum hydrocarbon as the sole carbon source and Iron III oxide and manganese IV oxide as electron acceptors) aimed at promoting the growth of petroleum hydrocarbon degraders that are also electro chemically active and are facultative anaerobes. Result from both analysis gives more insight than either of the two on which of the isolated microorganisms if selected had more potentials in enhancing the BES process that facilitated faster degradation of the petroleum hydrocarbon pollutants.

3.4.2 Groundwater

The metagenomics result of the bacterial community in the petroleum hydrocarbon polluted groundwater at the phylum level detected Proteobacteria to be the most dominant bacterial phylum as shown in **Figure 3.4.** With a percentage abundance of 59.327%, this phylum had more than half of the total bacterial abundance in the petroleum hydrocarbon polluted groundwater as shown in the Operational Taxonomy Units (OTUs) in **Figure 3.6**. The dominance of this phylum in the prolonged polluted petroleum hydrocarbon groundwater of the Niger-Delta region aligns with other previous studies. In petroleum polluted groundwater, Proteobacteria was reported to be the predominant phylum with as high as 52.60-91.65%

(Ma et al., 2021) and 29.3%-85.7% (Chen et al., 2022) of the total bacterial abundance. Although Proteobacteria was 59.327% of the total bacterial abundance as shown in the metagenomics analysis in **Figure 3.4,** the use of the screening pressures (anaerobic growth condition, petroleum hydrocarbon as the sole carbon source and Iron III oxide and manganese IV oxide as electron acceptors) in the two culture-based approaches can be seen to have promoted the growth and isolation of species belonging to this phylum more. Hence, increasing the percentage of the isolated species belonging to this phylum to a 100% and 66.6% as shown in **Table 3.1 and 3.2**. Similarly, at the genus level in **Figure 3.8** Pseudomonas was not among the top 10 genus but the use of the screening pressures promoted the growth and isolation of *Pseudomonas sp.* known to be electro active and a petroleum hydrocarbon degrader in **Table 3.1 and 3.2**. From the information provided by both metagenomics and culture-based approaches, these provide us with the insight of the most abundant microorganisms in the polluted groundwater and the influence of how selection pressures can promote the growth of potential microorganisms of interest. Information from both analysis gives more information on which of the isolated microorganisms if selected may have more potentials in enhancing the BES process than either of the two analyses. With the total number of 9 isolates from the two culture-based approaches used as shown on **Table 3.1 and 3.2**, the enrichment medium method to general medium method had a total of 6 isolates (66.666%) that are distinctive from general medium method to enrichment medium method with 3 isolates (33.333%). This is an indication that a combination of two culture-based approach can increase the number of potential microorganisms of interest being isolated. Details of the NCBI blast results for these pure isolates can be found on **Appendix 3.** Further studies aimed at selecting the most effective isolates that formed the members of the microcosms that were integrated to BES were carried out. This included the determination of

each isolate's electrochemical activity, biosurfactant production ability and either a facultative or strict anaerobe.

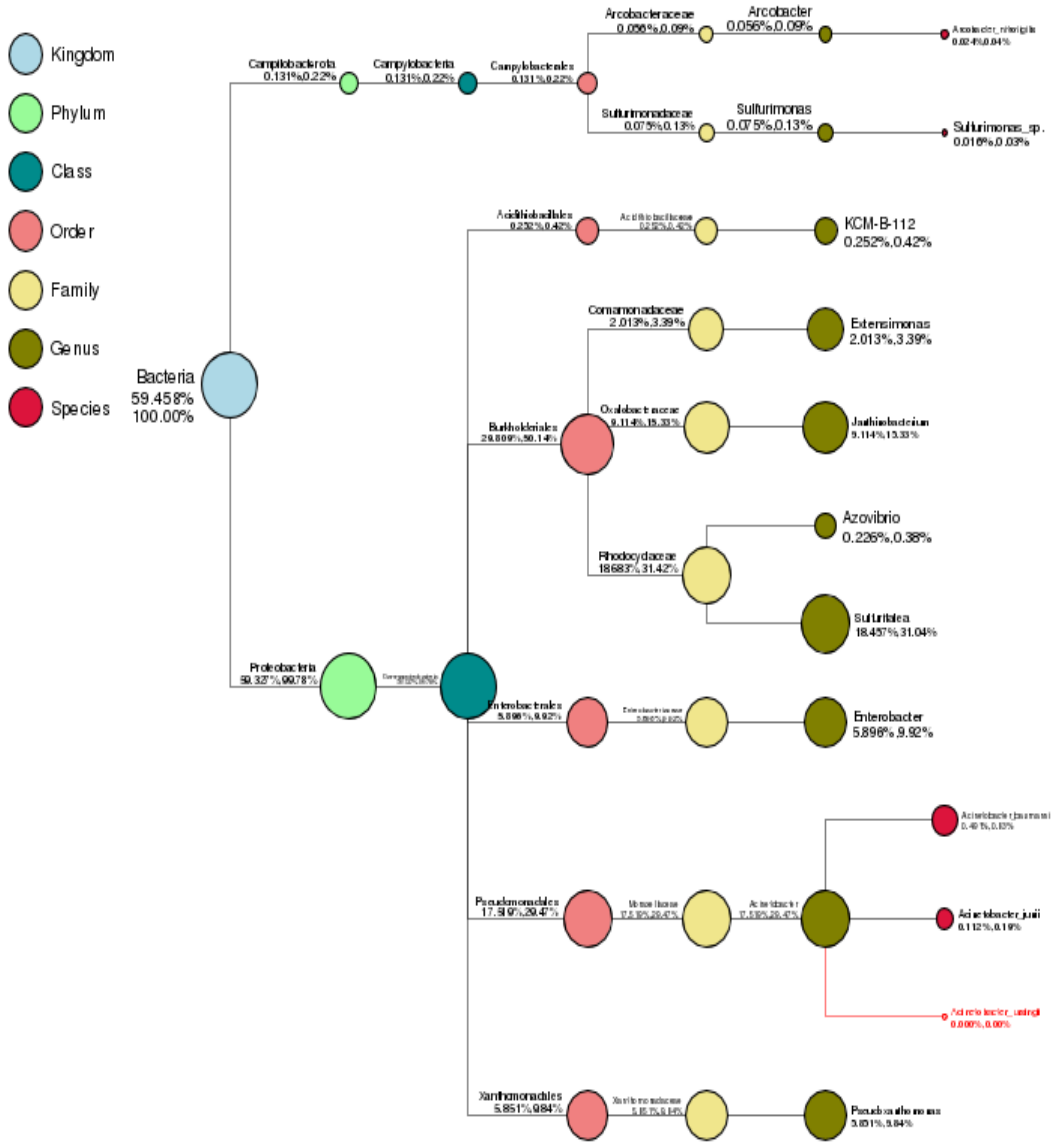


Figure 3.4 Taxonomy tree of the petroleum hydrocarbon polluted groundwater samples collected from the Niger-Delta region of Nigeria. Notes: different colours represent different taxonomic ranks. The size of circles represents the relative abundance of species. The first number below the taxonomic names (Kingdom, phylum, class, order, family, genus and species) represents the percentage in the whole taxon, while the second number represents the percentage in the selected taxon.

Table 3.1 16s rRNA result of unknown pure colonies isolated from petroleum hydrocarbon polluted groundwater showing the highly similar species and their respective taxonomy isolated using a general medium method to enrichment medium method. Percentage of Proteobacteria= 6 (100%)

Isolate code	**Scientific name of highly similar Species**	**Phylum**	**Class**	**order**	**Family**	**Genus**
2K	*Pseudomonas sp.*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
2N	*Pseudomonas libanensis*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
2O	*Pseudomonas sp.*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
2Q	*Pseudomonas sp. SCB32*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
2Q	*Pseudomonas sp. J380*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
2J	*Pseudomonas sp. SCB32*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas

Table 3.2 16s rRNA result of unknown pure colonies isolated from petroleum hydrocarbon polluted groundwater showing the highly similar species and their respective taxonomy that were isolated using a general medium method to enrichment medium method. Percentage of each Proteobacteria= 2 (66.6%) and Firmicutes= 1 (33.3%)

Sample code	**Scientific name of highly similar Species**	**Phylum**	**Class**	**order**	**Family**	**Genus**
5	*Pseudomonas sp. LCR71*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
A	*Pseudomonas azotoformans*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
1	*Clostridium senegalense JC122*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium

3.4.3 Soil

The metagenomics result of the bacterial community in the petroleum hydrocarbon polluted soil showed a relative significant abundance of species across the top 10 phylum in **Figure 3.5.** However, the phylum Proteobacteria was the predominant bacterial phylum with a bacterial abundance of 25.541% as shown in the Operational Taxonomy Units in **Figure 3.7**. The result is similar to previous studies that have reported the dominance of the Proteobacteria phylum in petroleum hydrocarbon polluted soil with 52.5–69.9% (Das et al., 2022), 44.4% (Bao et al., 2017) and 3.91-57.01% (Gao et al., 2022) of bacterial abundance. Although being the most dominant phylum with just about a quarter (25.541%) of the bacterial abundance as shown **Figure 3.7,** the use of the screening pressures (anaerobic growth conditions, use of petroleum hydrocarbon as the sole carbon source and Iron III oxide and manganese IV oxide as electron acceptors) in the two culture-based approaches promoted the growth and isolation of species belonging to the Proteobacteria phylum. This has resulted to an increase in the percentage of the isolated species belonging to this phylum to a 77.7% and 90% for the enrichment medium method to general medium method and general medium method to enrichment medium method respectively as shown in as shown in **Table 3.3 and 3.4.** Similarly, at the genus level in **Figure 3.8** Pseudomonas was not among the top 10 genus but the screening pressures promoted the growth and isolation of *Pseudomonas sp.* a genus known for electrochemical activity and a petroleum hydrocarbon degrader in **Table 3.3 and 3.4**. From the information provided by both metagenomics and culture-based approaches, metagenomics gives an insight on the most abundant microorganisms in the polluted soil while the culture-based approaches show the effectiveness of how a selection pressures can promote the growth of potential microorganisms of interest. Information from both analysis gives more insight than

either of the two on which of the isolated microorganisms if selected may have more potentials in enhancing the BES process. With the total number of 9 isolates from the two culture approaches used as shown on **Table 3.3 and 3.4**, the enrichment medium method to general medium method had a total of 9 isolates (47.36%) that are distinctive from general medium method to enrichment medium method with 10 isolates (52.363%). This is an indication that a combination of two culture-based approach (reversed approach) can increase the number of potential microorganisms of interest being isolated. Details of the NCBI blast results for these pure isolates can be found on **Appendix 3.** Further studies aimed at selecting the most effective isolates that formed the members of the microcosms that were integrated to BES were carried out. This included determining each isolate's electrochemical activity, biosurfactant production ability and either a facultative or strict anaerobe.

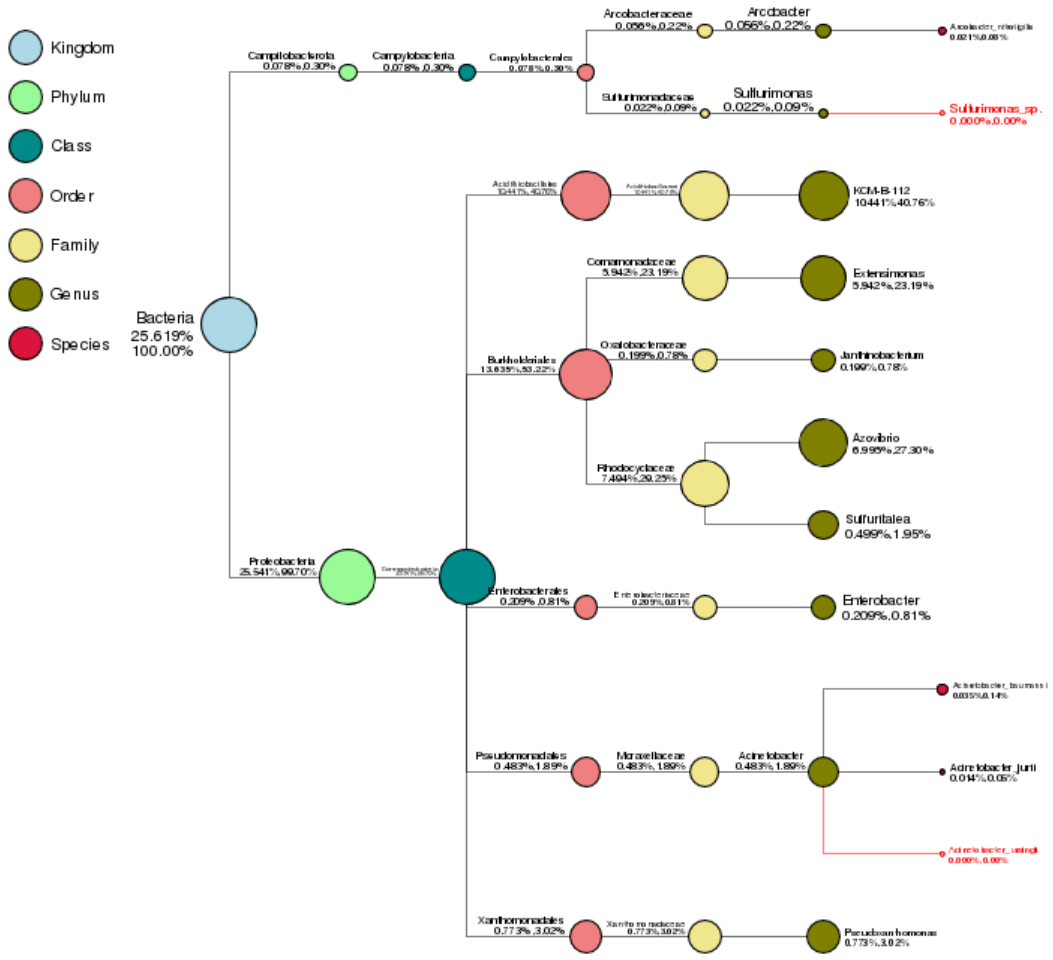


Figure 3.5 Taxonomy tree of the petroleum hydrocarbon polluted soil sample collected from the Niger-Delta region of Nigeria. Notes: different colours represent different taxonomic ranks. The size of circles represents the relative abundance of species. The first number below the taxonomic names (Kingdom, phylum, class, order, family, genus and species) represents the percentage in the whole taxon, while the second number represents the percentage in the selected taxon.

Table 3.3 16s rRNA result of unknown pure colonies isolated from petroleum hydrocarbon polluted soil showing the highly similar species and their respective taxonomy isolated using a general medium method to enrichment medium method. The percentage of Proteobacteria= 9 (90%) and Firmicutes= 1 (10%).

Sample code	**Scientific name of highly similar Species**	**Phylum**	**Class**	**order**	**Family**	**Genus**
2B	*Pseudomonas sp. CSJ-3*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
B	*Clostridium beijerinckii*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium
J	*Ewingella americana*	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Ewingella
F	*Pseudomonas paralactis*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
9	*Ewingella americana*	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Ewingella
E	*Pseudomonas fluorescens*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
8	*Enterobacter ludwigii*	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Enterobacter
10	*Ewingella americana*	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Ewingella
M	*Pseudomonas azotoformans*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
C	*Pseudomonas azotoformans*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas

Table 3.4 16s rRNA result of unknown pure colonies isolated from petroleum hydrocarbon polluted soil showing the highly similar species and their respective taxonomy isolated using an enrichment medium method to general medium method. Percentage of Proteobacteria= 7 (77.7%), Fiirmicutes=1 (11.1%), Uncultured bacterium = 1 (11.1%)

isolate code	**Scientific name of highly similar Species**	**Phylum**	**Class**	**order**	**Family**	**Genus**
1E	*Ewingella americana*	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Ewingella
1F	*Microvirgula aerodenitrificans*	Proteobacteria	Betaproteobacteria	Neisseriales	Chromobacteriaceae	Microvirgula
2M	*Microvirgula aerodenitrificans*	Proteobacteria	Betaproteobacteria	Neisseriales	Chromobacteriaceae	Microvirgula
2S	*Microvirgula aerodenitrificans*	Proteobacteria	Betaproteobacteria	Neisseriales	Chromobacteriaceae	Microvirgula
1G	*Rahnella sp. UIWRF1115*	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Rahnella
1J	*uncultured bacterium*	uncultured bacterium				
1D	*Ewingella americana*	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae	Ewingella
2P	*Pseudomonas sp.*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
1I	*Clostridium sp. Cd6*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium

3.4.4 Sediment

The metagenomics result of the bacterial community in the petroleum hydrocarbon polluted sediment showed Campilobacterota as the predominant phylum as shown in **Figure 3.7** and a 19.4% as shown in the Operational Taxonomy Units in **Figure 3.8**. Although Proteobacteria has been reported to be a predominant Phylum in some petroleum hydrocarbon sediment studies with 55.7–78.0% (Ma et al., 2021), 54% (Korlevic et al., 2015) and 40.2% (Phulpoto et al., 2021) they also reported a significant presence of other phyla that include Firmicutes, Bacteroidetes and Cyanobacteria etc. Although Campilobacterota is the most dominant phylum, it is very insightful to observe how the use of the screening pressures (anaerobic growth conditions, use of petroleum hydrocarbon as the sole carbon source and Iron III oxide and manganese IV oxide as electron acceptors) in the two culture-based approaches promoted mostly the growth and isolation of species belonging to the phylum Proteobacteria and Firmicutes as shown in **Table 3.5 and table 3.6**. It is very revealing to see how using different culturing approaches while maintaining the same selection pressures and samples can favour the growth of species from entirely different phylum. While the general medium method to enrichment medium method promoted the growth of Firmicutes to as high as 83.3%, the enrichment medium method to general medium method promoted the growth of Proteobacteria to 70% as shown in **Table 3.5 and table 3.6**. Similarly, at the genus level in **Figure 3.8** Pseudomonas and Clostridium were not among the top 10 genus but the screening pressures promoted the growth and isolation of *Pseudomonas sp.* and *Clostridium sp.* as shown in **Table 3.5 and 3.6**. From the information provided by both metagenomics and culture-based approaches, these give insight on the most abundant microorganisms in the polluted soil and the effectiveness of how selection pressures can promote the growth of

potential microorganisms of interest. Information from both analysis gives more insight than either of the two on which of the isolated microorganisms if selected may have more potentials in enhancing the BES process. With the total number of 9 isolates from the two culture approaches used as shown on **Table 3.3 and 3.4**, the enrichment medium method to general medium method had a total of 10 isolates (62.5%) that are distinctive from general medium method to enrichment medium method with 6 isolates (37.5%). This is an indication that a combination of two culture-based approach (reversed approach) can increase the number of potential microorganisms of interest being isolated. Details of the sequence and the NCBI blast results for these pure isolates can be found on **Appendix 3.** Further studies aimed at selecting the most effective isolates that formed the members of the microcosms that was integrated to BES were carried out. This included determining each isolate's electrochemical activity, biosurfactant production ability and either a facultative or strict anaerobe.

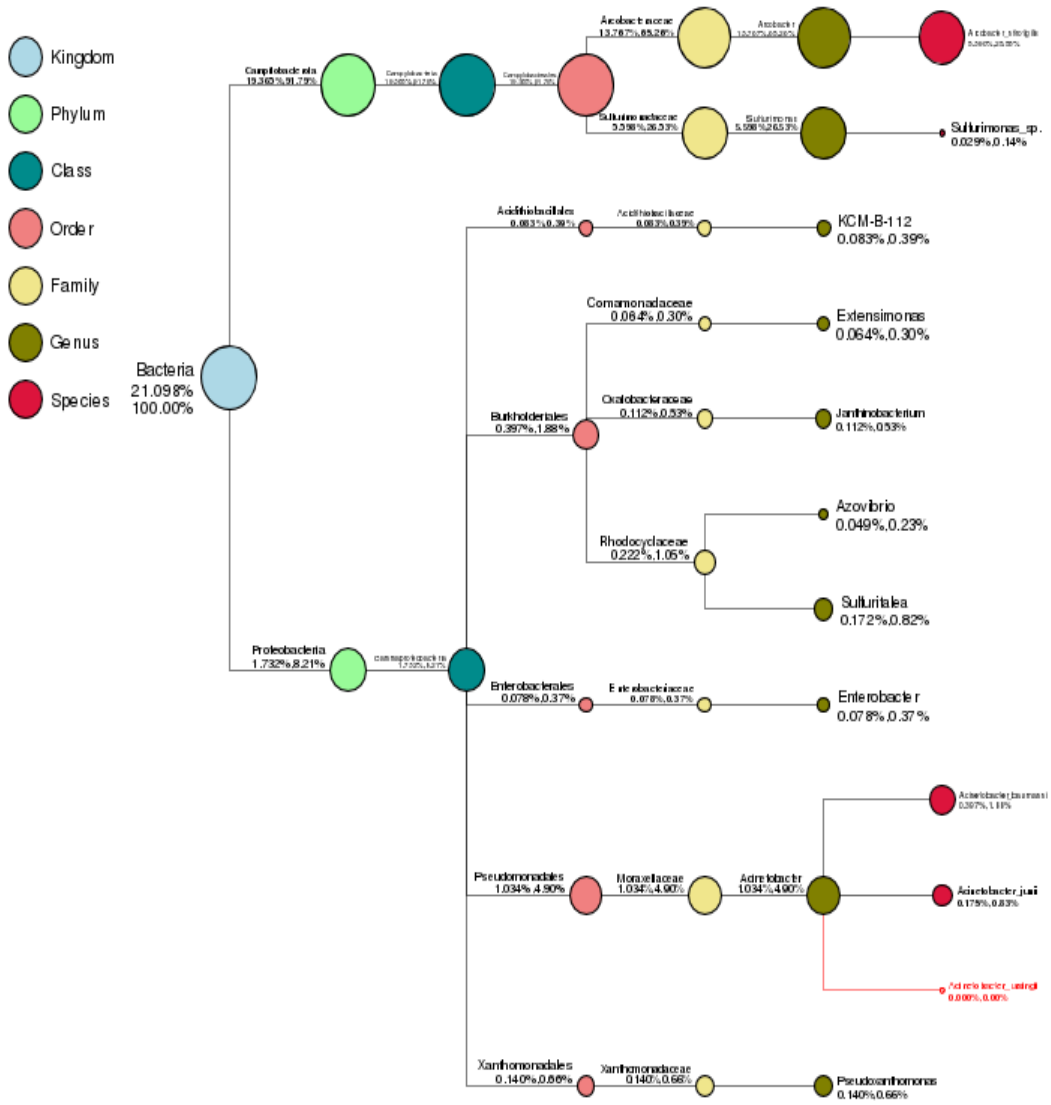


Figure 3.6 Taxonomy tree of the petroleum hydrocarbon polluted sediment sample collected from the Niger-Delta region of Nigeria. Notes: different colours represent different taxonomic ranks. The size of circles represents the relative abundance of species. The first number below the taxonomic names (Kingdom, phylum, class, order, family, genus and species) represents the percentage in the whole taxon, while the second number represents the percentage in the selected taxon.

Table 3.3 16s rDNA result of unknown pure colonies isolated from petroleum hydrocarbon polluted sediment showing the highly similar species and their respective taxonomy isolated using an enrichment medium method to general medium method. Percentage of Firmicutes= 5 (83.3%) and Proteobacteria= 1 (16.6%)

Isolate code	**Scientific name of highly similar Species**	**Phylum**	**Class**	**order**	**Family**	**Genus**
2A	*Pseudomonas azotoformans*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
2D	*Clostridium butyricum*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium
1B	*Clostridium sp. Marseille-P8840*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium
2U	*Lacrimispora saccharolytica*	Firmicutes	Clostridia	Eubacteriales	Lachnospiraceae	Lacrimispora
G	*Clostridium butyricum*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium
2E	*Clostridium butyricum*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium

Table 3.4 16s rRNA result of unknown pure colonies isolated from petroleum hydrocarbon polluted sediment showing the highly similar species and their respective taxonomy isolated using an enrichment medium method to general medium method. Percentage of Proteobacteria= 7 (70%), Fermicutes=2 (20%) and Bacteria incertae sedis=1 (10%)

Isolate code	**Scientific name of highly similar Species**	**Phylum**	**Class**	**order**	**Family**	**Genus**
1R	*Pseudomonas sp.*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
1Q	*Pseudomonas sp. sw6*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
1L	*Clostridium butyricum*	Firmicutes	Clostridia	Eubacteriales	Clostridiaceae	Clostridium
1O	*Pseudomonas sp.*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
2X	*Alphaproteobacteria bacterium*	Proteobacteria	Alphaproteobacteria	unclassified Alphaproteobacteria		
2F	*Pseudomonas chengduensis*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
1M	*Lacrimispora celerecrescens*	Firmicutes	Clostridia	Eubacteriales	Lachnospiraceae	Lacrimispora
2V	*Nanobacterium sp. Persian TH hmb*	Bacteria incertae sedis	Nanobacterium	unclassified Nanobacterium		
2T	*Pseudomonas sp.*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
1N	*Pseudomonas alcaliphila*	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas

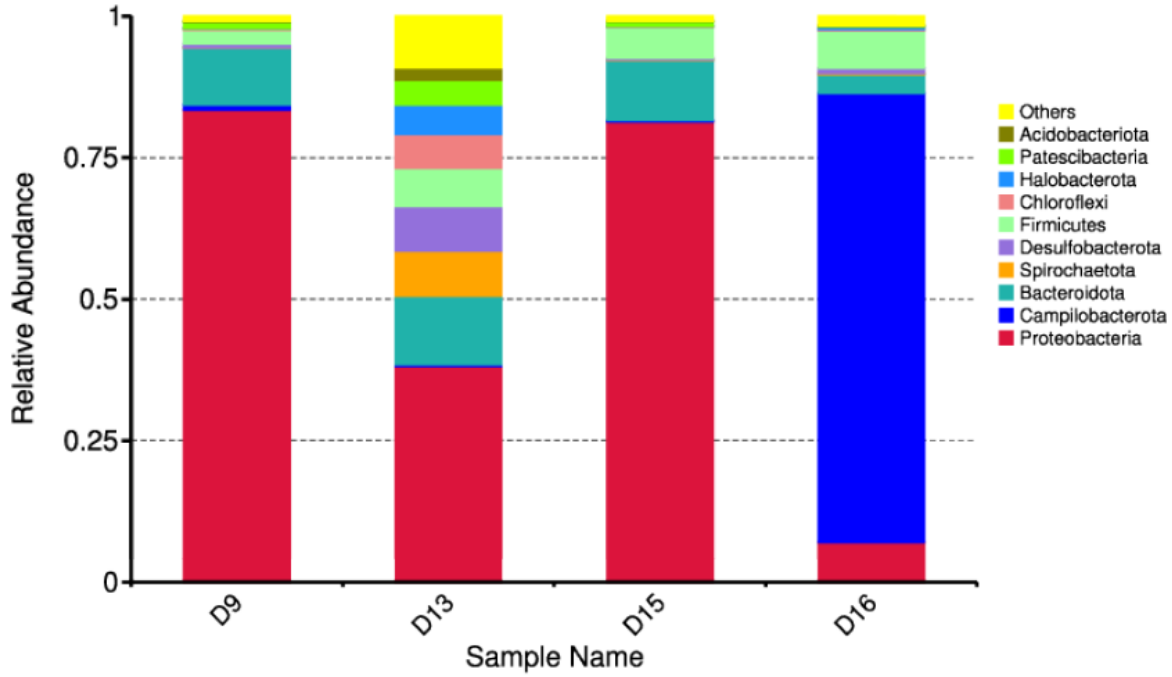


Figure 3.7 OTUs showing the relative abundance of bacterial phylum in petroleum polluted matrices (groundwater (D9 and D15) soil (13), sediment (D16)) collected from the Niger-Delta region of Nigeria. Note: that the polluted groundwater was duplicated for the purpose of analysis confirmation; Y-axis represents "Relative Abundance" and X-axis represents "Samples Name". "Others" represents a total relative abundance of the rest phyla besides the top 10 phyla.

3.5 Electrochemical activity of isolated pure colonies

3.5.1 Overview

BES that utilises electrochemically active microorganisms as biocatalysts has found acceptability and use in bioremediation due to its ability to facilitates continuous electrons transfer, significantly enhance bioremediation, shorten remediation time and reduce cost (Wang et al., 2020). Several studies have reported the use of this technology for the degradation of petroleum hydrocarbon (Wei et al. 2015; Adelaja et al., 2017; Zhang et al. 2010; Logan 2009). These electro active microorganisms have the ability to release electrons generated by substrates utilization under anaerobic respiration to electron acceptors through a transmembrane electron transfer (TET) in various

bioelectrochemical systems. These electrons are transported to the extracellular cytochromes through a transmembrane

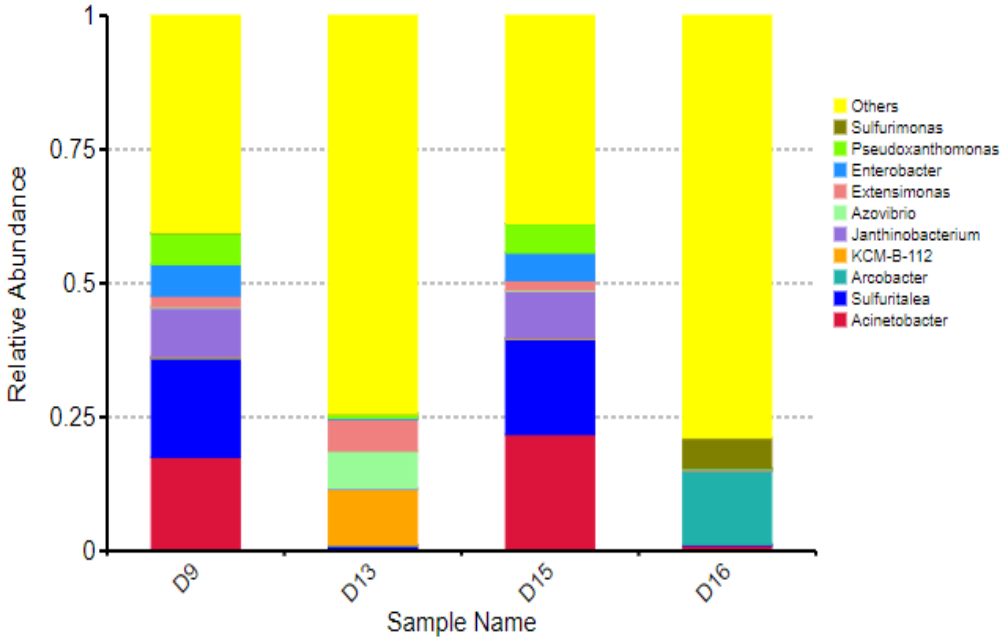


Figure 3.8 OTUs showing the relative abundance of bacterial genus in petroleum polluted matrices (groundwater (D9 and D15) soil (13), sediment (D16)) collected from the Niger-Delta region of Nigeria. Note: that the polluted groundwater (D9 and D15) are duplicates; Y-axis represents "Relative Abundance" and X-axis represents "Samples Name". "Others" represents a total relative abundance of the rest phyla besides the top 10 phyla.

electron transfer, this entire process is characterised into electron input, transmembrane delivery and the extracellular release of the electrons. Because of the entire process involved, the cell structures of the electro active microorganisms play a key role on how efficient this pathway works. The mechanism of the process depends on either an electroactive bacterium is gram positive or negative. Structurally, gram negative Electro Active Bacteria (EAB) have both an inner and outer membrane that are separated by a periplasmic space. When electrons are generated from the oxidation of organic substrates, they are usually attached with NADH the reduced form of nicotinamide adenine dinucleotide. For extracellular electron transfer to happen in gram negative electroactive bacteria, these electrons have to flow from

the NADH and pass through the inner membrane, periplasmic space and finally the outer membrane that releases them to the external electron acceptor (Xiao and Yu., 2020). On the other hand, gram positive electroactive bacteria with a thicker cell wall (20-80 nm) that is made up of peptidoglycan (Lusk., 2019) has a different pathway that results in extracellular electron transfer. Due to the single-membrane architecture of the electroactive gram-positive bacteria, they are able to achieve transmembrane electron transfer (TET) by completing transfer steps unlike the gram negative with an inner membrane. While TET is faster in the gram-positive bacteria, the major restricting factor to the complete process is the thick nonconducting cell wall that severely limits the Extracellular Electron Transfer (EET) to an external electron acceptor (Tian., 2019; You., 2018). This restrictions of EET by the thick cell wall of gram positive EAB have been considered as the reason for its less efficiency compared to gram negative EAB like Shewanella and Geobacter (Pankratova., 2019). The sample code is a unique code assigned to each isolate that is morphologically different from any other isolate, scientific name of highly similar Species to each isolate and the results of electrochemical activity of each pure isolate against a negative control and subsequently a positive control (*Shewanella oneidensis)* are presented and discussed below. EAB being the biocatalyst in BES was considered necessary to assess each pure isolates bacteria in section **3.4**

3.5.2 Comparison of isolates with negative control

3.5.2.1 Groundwater

The isolates from the polluted petroleum hydrocarbon groundwater exhibited significant electrochemical activity compared to the negative control. Out of the 9 organisms isolated from both methods in **Table 3.7 and 3.8**, about 77.78% of these isolates had a peak current

greater than that of the negative control. Among the 77.78%, 66.67% of them had a peak current that was more than 100% greater than the negative control's peak current. While 5 out of the 6 isolates representing a total of 83.33% in **Table 3.8** had a peak current greater than the negative control, those in **Table 3.7** had 66.67% of isolates peak current above that of the negative control. While those in **Table 3.8** are all closely related to the genus Pseudomonas known to be gram negative organisms, **Table 3.7** had 2 Pseudomonas and Clostridium. Comparing the peak currents isolates from both table that are above that of negative control, it can be observed that a higher percentage of the gram-negative organisms had a peak current greater than that of the negative control. This aligns with previous observations that generally, gram-negative bacteria tend to be more efficient at extracellular electron transfer than gram positive bacteria (Pankratova., 2019). A voltammogram of one of the bacteria isolated from the petroleum hydrocarbon polluted groundwater is presented in **Figure 3.9.**

Table 3.5 Peak current and potential at a scan rate of 10 mV/s of bacteria isolated from petroleum hydrocarbon polluted groundwater using the general medium method to enrichment medium method. Note: Scientific name of highly similar Species are blast results of isolates 16s rRNA, PCI (Peak current of isolates), PCNC (Peak current of negative control), Percentage % is the percentage of isolates peak current above or below the peak current of negative control.

Isolate code	**Scientific name of highly similar Species**	**Peak current (µA)**	**Potential (V)**	**PCI-PCNC**	**Percentage (%)**
5	*Pseudomonas sp. LCR71*	989.583	0.084	825.416	502.8
A	*Pseudomonas azotoformans*	864.167	0.074	700	426.4
1	*Clostridium senegalense JC122*	162.5	0.242	-1.667	-1.0
Negative control	N\A	164.167	-0.004	N/A	N/A

Table 3.6 Peak current and potential at a scan rate of 10 mV/s of bacteria isolated from petroleum hydrocarbon polluted groundwater using the enrichment medium method to general medium method. Note: Scientific name of highly similar Species are blast results of isolates 16s rRNA, PCI (Peak current of isolates), PCNC (Peak current of negative control), Percentage % is the percentage of isolates peak current above or below the peak current of negative control

Isolate code	**Scientific name of highly similar Species**	**Peak current (µA)**	**Potential (V)**	**PCI-PCNC**	**Percentage (%)**
2K	*Pseudomonas sp.*	712.25	-0.018	548.083	333.8
2N	*Pseudomonas libanensis*	1028.33	0.25	864.163	526.4
2O	*Pseudomonas sp.*	159.125	0.1	-5.042	-3.0
2Q	*Pseudomonas sp. SCB32*	272.083	0.04	107.916	65.7
2W	*Pseudomonas sp. J380*	947.5	0.048	783.333	477.2
2J	*Pseudomonas sp. SCB32*	575	0.062	410.833	250.3
Negative control	N\A	164.167	-0.004	N/A	N/A

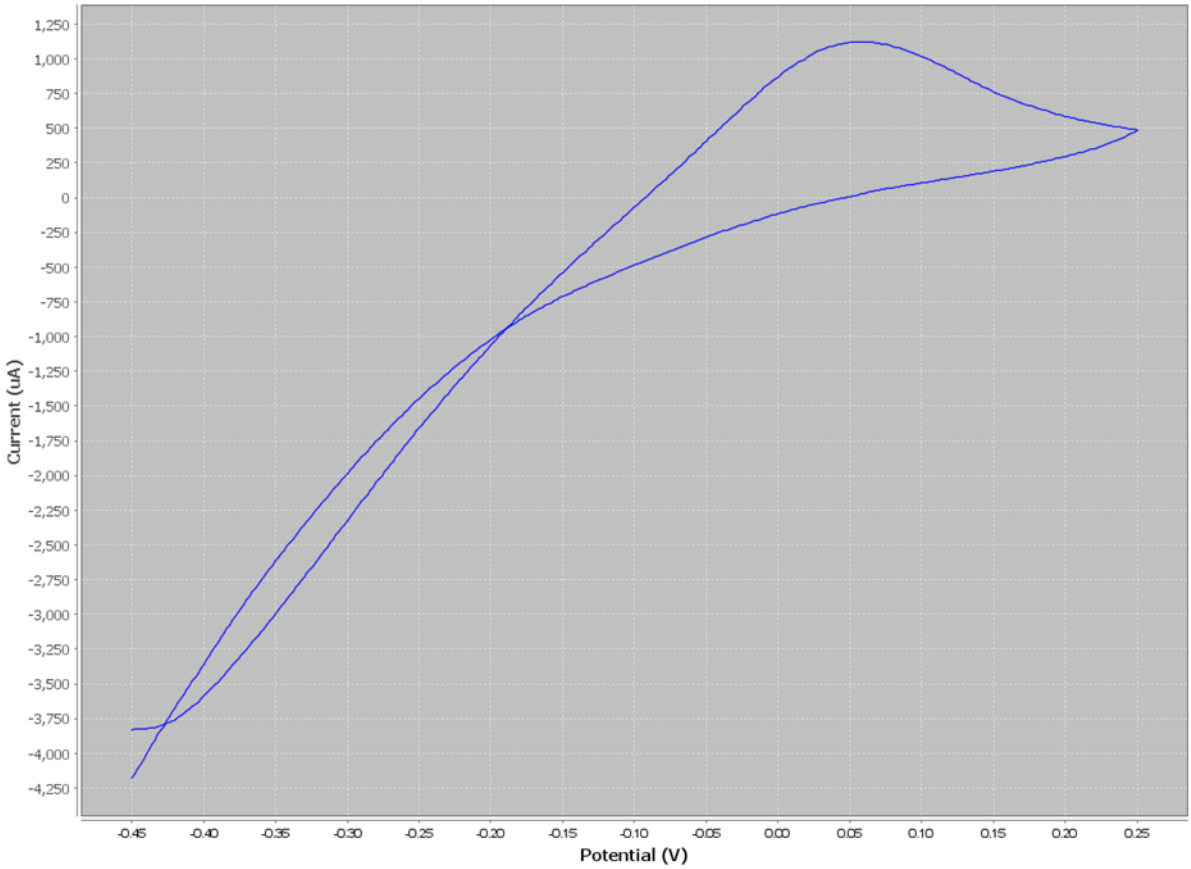


Figure 3. 9 Voltammogram at a scan rate of 10 mV/s of a bacterium isolated from petroleum hydrocarbon polluted groundwater.

3.5.2.2 Soil.

Generally, the isolates from the polluted petroleum hydrocarbon soil exhibited significant electrochemical activity compared to the negative control. 16 out of the 19 bacteria isolates from both methods in **Table 3.9 and 3.10** representing 84.2% had a peak current greater than that of the negative control. Among the 84.2%, 73.68% of them had a peak current that was 100% greater than the peak current of the negative control. While 8 out of the 9 isolates representing 88.89% of the isolates in **Table 3.9** had a peak current greater than the negative control, those in **Table 3.10** had 80% (8 out of 10) of isolates peak current above that of the negative control. The isolates from both tables are predominantly closely related to gram-negative bacteria there by exhibiting higher electrochemical activity far above the negative control. The result generally aligns with previous observations that gram-negative bacteria tend to have a more efficient at extracellular electron transfer because of the absence of the thick cell wall found in gram positive bacteria (Pankratova., 2019). Isolates **B** in **Table 3.10** closely related to *Clostridium beijerinckii* a supposedly gram-positive bacterium exhibited high electro activity an indication of an efficient EET process. It is important to note that further studies such as whole genome analysis was carried out to ascertain the identity of all isolates that exhibited efficient electrochemical activity. A voltammogram of one of the bacteria isolated from the petroleum hydrocarbon polluted soil is presented in **Figure 3.10.**

3.5.2.3 Sediment

Generally, the isolates from the polluted petroleum hydrocarbon sediment exhibited significant high electrochemical activity compared to the negative control. 87.5% (14 out of 16) isolates from both methods in **Table 3.11 and 3.12**, had a peak current greater than that of the negative control. Among the 87.5%, 68.75% of them had a peak current that was 100% greater than that of the negative control. A 100% of the isolates in **Table 3.12** that are

predominantly related to gram-negative bacteria had a peak current greater than the negative control. On the other hand, those in **Table 3.11** predominantly gram positive had 66.67% of isolates' peak current above that of the negative control. Comparing the peak currents of both table that are above that of negative control, it can be observed that a 33.3% of isolates closely related to gram-negative in **Table 3.12** had a peak current greater than that

Table 3.7 Peak current and potential at a scan rate of 10 mV/s of a bacteria isolated from petroleum hydrocarbon polluted soil using the enrichment medium method to general medium method. Note: Scientific name of highly similar Species are blast results of isolates 16s rRNA, PCI (Peak current of isolates), PCNC (Peak current of negative control), Percentage % is the percentage of isolates peak current above or below the peak current of negative control

Isolate code	**Scientific name of highly similar Species**	**Peak current (µA)**	**Potential (V)**	**PCI-PCNC**	**Percentage (%)**
1E	*Ewingella americana*	518	0.082	354	216
1F	*Microvirgula aerodenitrificans*	555	0.080	390	238
2M	*Microvirgula aerodenitrificans*	607	0.250	443	270
2S	*Microvirgula aerodenitrificans*	544	0.098	380	232
1G	*Rahnella sp. UIWRF1115*	982	0.048	818	498
1J	*uncultured bacterium*	423	0.096	259	158
1D	*Ewingella americana*	438	0.250	274	167
2P	*Pseudomonas sp.*	131	0.250	-33	-20
1I	*Clostridium sp. Cd6*	196	0.052	32	20
Negative control	N\A	164	-0.004	N/A	N/A

Table 3.8 Peak current and potential at a scan rate of 10 mV/s of a bacteria isolated from petroleum hydrocarbon polluted soil using the general medium method to enrichment

medium method. Note: Scientific name of highly similar Species are blast results of isolates 16s rRNA, PCI (Peak current of isolates), PCNC (Peak current of negative control), Percentage % is the percentage of isolates peak current above or below the peak current of negative control.

Isolate code	**Scientific name of highly similar Species**	**Peak current (µA)**	**Potential (V)**	**PCI-PCNC**	**Percentage (%)**
2B	*Pseudomonas sp. CSJ-3*	840	0.074	676	412
B	*Clostridium beijerinckii*	1548	0.078	1384	847
J	*Ewingella americana*	168	0.052	4	2.5
F	*Pseudomonas paralactis*	467.5	0.25	303	185
9	*Ewingella americana*	425	0.042	261	150
E	*Pseudomonas fluorescens*	48	0.25	-116	-70.8
8	*Enterobacter ludwigii*	89	0.028	-75	-46
10	*Ewingella americana*	434	0.25	270	164
M	*Pseudomonas azotoformans*	680	0.068	516	315
C	*Pseudomonas azotoformans*	1015	0.058	851	518
Negative control	N\A	164	-0.004	N/A	N/A

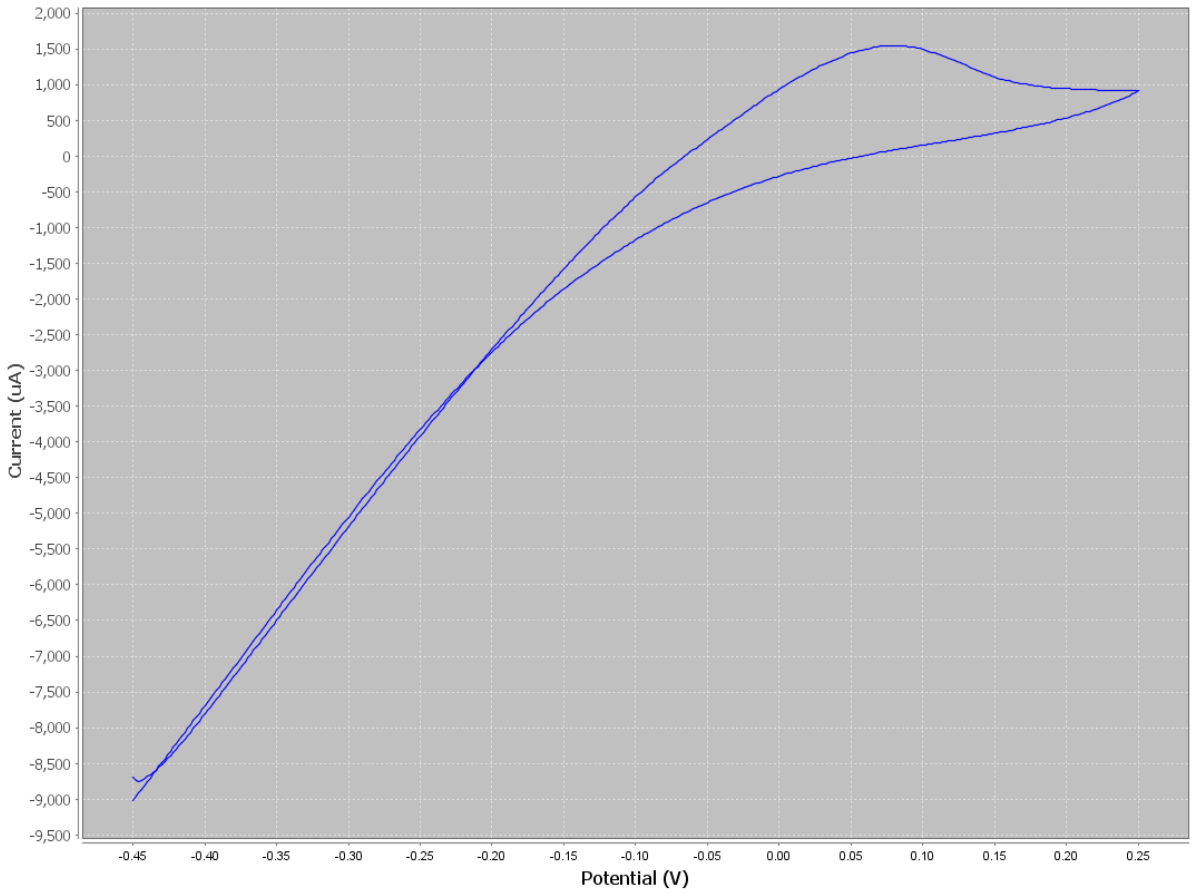


Figure 3.10 Voltammogram at a scan rate of 10 mV/s of a bacterium isolated from petroleum hydrocarbon polluted soil.

of the negative control compared to those closely related to gram-positive bacteria in **Table 3.11**. This aligns with previous observations that gram-negative bacteria generally tend to be more efficient at extracellular electron transfer than gram positive bacteria (Pankratova., 2019). A voltammogram of one of the bacteria isolated from the petroleum hydrocarbon polluted sediment is presented in **Figure 3.11**.

Table 3.9 Peak current and potential at a scan rate of 10 mV/s of a bacteria isolated from petroleum hydrocarbon polluted sediment using the general medium method to enrichment medium method. Note: Scientific name of highly similar Species are blast results of isolates 16s rRNA, PCI (Peak current of isolates), PCNC (Peak current of negative control), Percentage % is the percentage of isolates peak current above or below the peak current of negative control.

Isolate code	Scientific name of highly similar Species	Peak current (µA)	Potential (V)	PCI-PCNC	Percentage (%)
2A	*Pseudomonas azotoformans*	338.417	0.04	174.25	106.1
2D	*Clostridium butyricum*	226	0.042	61.833	37.7
1B	*Clostridium sp. Marseille-P8840*	150.833	0.034	-13.334	-8.1
2U	*Lacrimispora saccharolytica*	412.5	0.25	248.333	151.2
G	*Clostridium butyricum*	580	0.15	415.833	253.2
2E	*Clostridium butyricum*	143.625	0.036	-20.542	-12.5
Negative control	N\A	164.167	-0.004	N/A	N/A

Table 3.10 Peak current and potential at a scan rate of 10 mV/s of a bacteria isolated from petroleum hydrocarbon polluted sediment using the enrichment medium method to general medium method. Note: Scientific name of highly similar Species are blast results of isolates 16s rRNA, PCI (Peak current of isolates), PCNC (Peak current of negative control), Percentage % is the percentage of isolates peak current above or below the peak current of negative control.

Isolate code	Scientific name of highly similar Species	Peak current (µA)	Potential (V)	PCI-PCNC	Percentage (%)
1R	*Pseudomonas sp.*	1120.47	0.25	956.303	582.5
1Q	*Pseudomonas sp. sw6*	1493.75	0.058	1329.583	809.9

1L	*Clostridium butyricum*	219.167	0.25	55	33.50
1O	*Pseudomonas sp.*	1206.042	0.046	1041.875	634.6
2X	*Alphaproteobacteria bacterium*	466.25	0.25	302.083	184.0
2F	*Pseudomonas chengduensis*	366.875	0.25	202.708	123.4
1M	*Lacrimispora celerecrescens*	164.583	0.25	0.416	0.3
2V	*Nanobacterium sp. Persian TH hmb*	873.042	0.072	708.875	431.8
2T	*Pseudomonas sp.*	713.958	0.064	549.791	334.9
1N	*Pseudomonas alcaliphila*	663.333	0.062	499.166	304.1
Negative control	N\A	164.167	-0.004	N/A	N/A

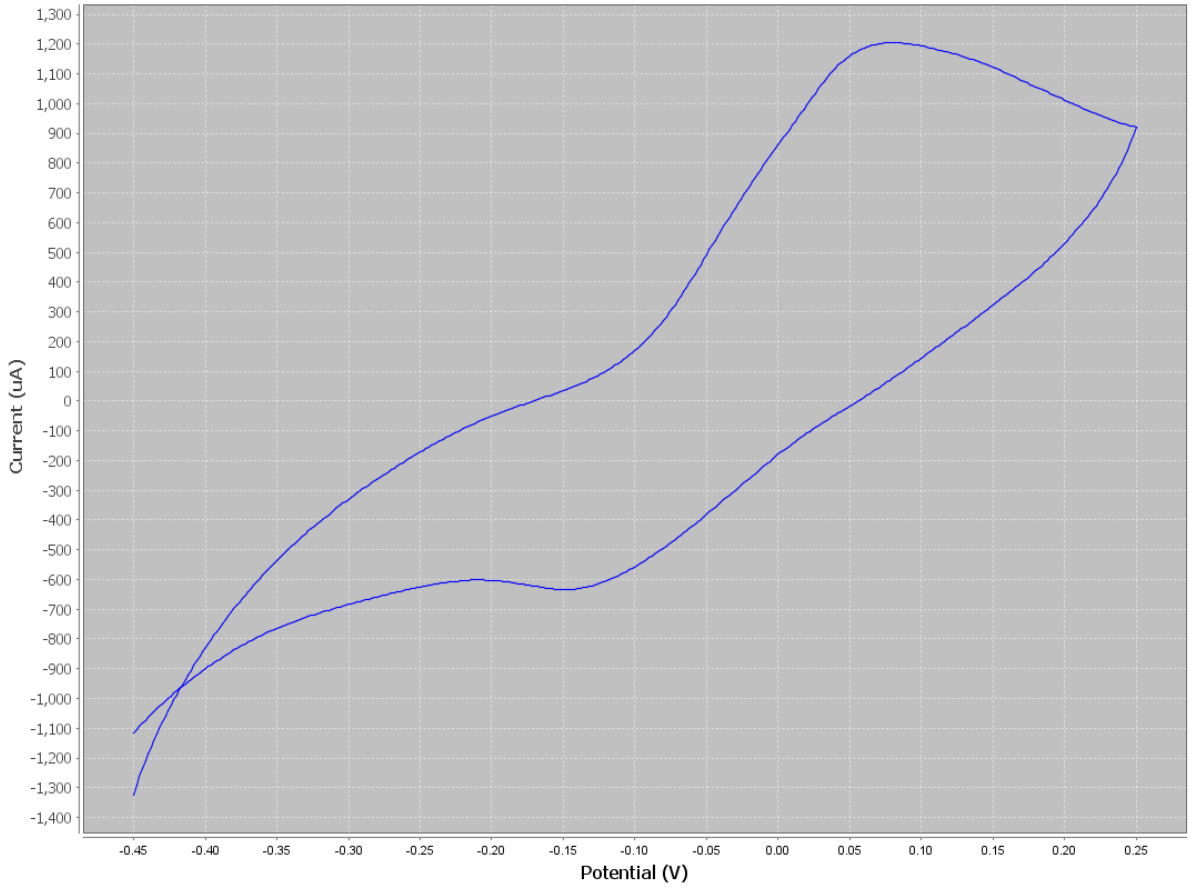


Figure 3.11 Voltammogram at a scan rate of 10 mV/s of a bacterium isolated from petroleum hydrocarbon polluted sediment.

From the results above, the electrochemical activity of the isolates across the three polluted petroleum hydrocarbon matrices had a significant number of isolates whose peak current was significantly higher than of the negative control. Those with peak current that was equal to or greater than 100% of the negative control's peak current was selected. Across the three matrices, soil had the highest number of isolates representing 74% of its total number whose peak current was more than 100% that of the negative control, this was followed by sediment and groundwater with 69% and 67% respectively.

3.5.3 Comparison of isolates with positive control (*Shewanella oneidensis*).

Having established the electrochemical activity of these isolates, those with a peak current that was 100% and above that of the negative control were selected to be members of the microcosms that were used for subsequent experiment for each matrix. However, it was important to compare these with a positive control (*Shewanella oneidensis*) to have an insight to their electrochemical capacity and their possible performance in subsequent experiments using MFCs. *S. oneidensis* is a known model for power generation owing to their electrochemical activity in MFC (Wang et al., 2015). The results of the outcome are presented

below.

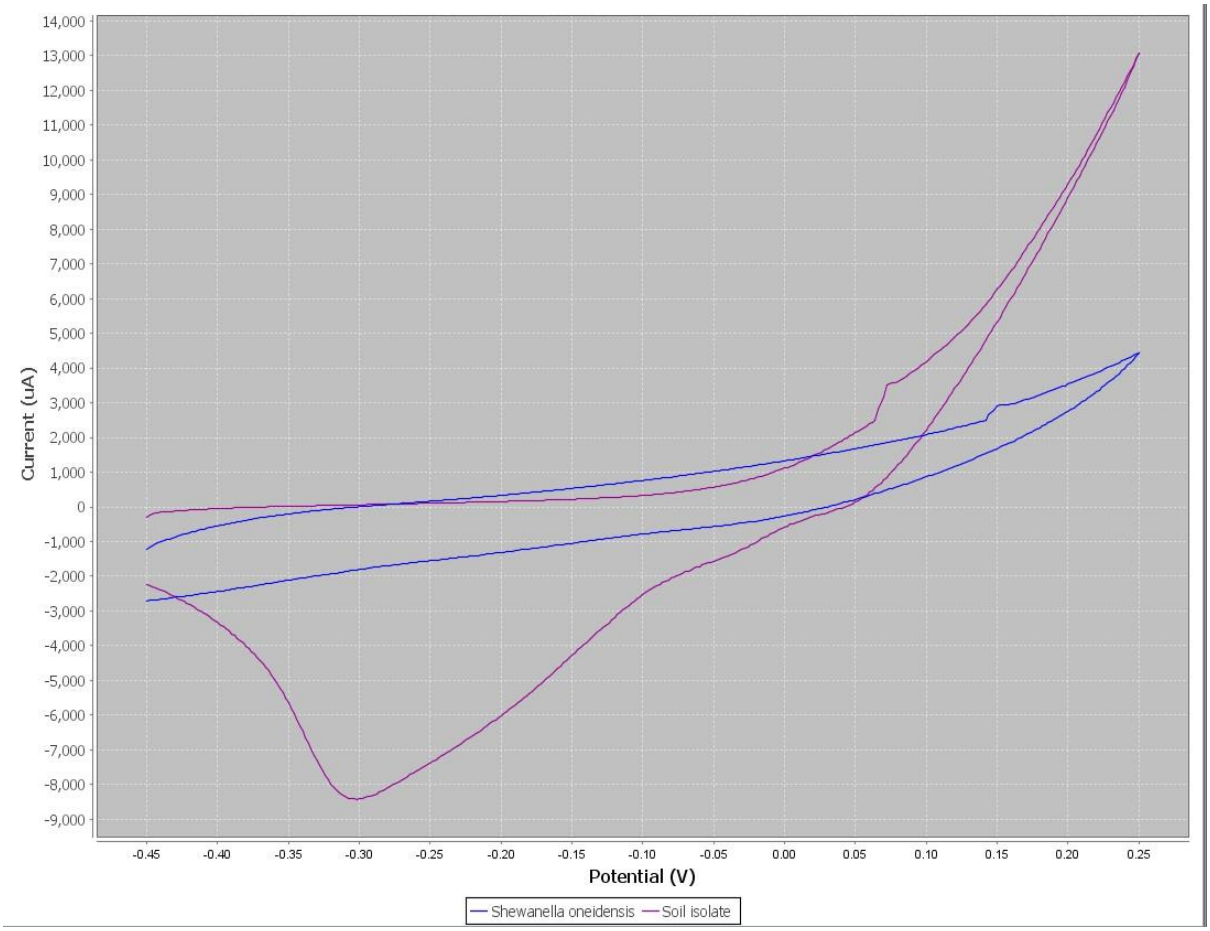


Figure 3.12 Voltammogram of S. oneidensis in blue colour overlapped by one of the bacteria in pink colour that was isolated from the polluted petroleum hydrocarbon soil at a scan rate of 10 mV/s.

3.5.3.1 soil

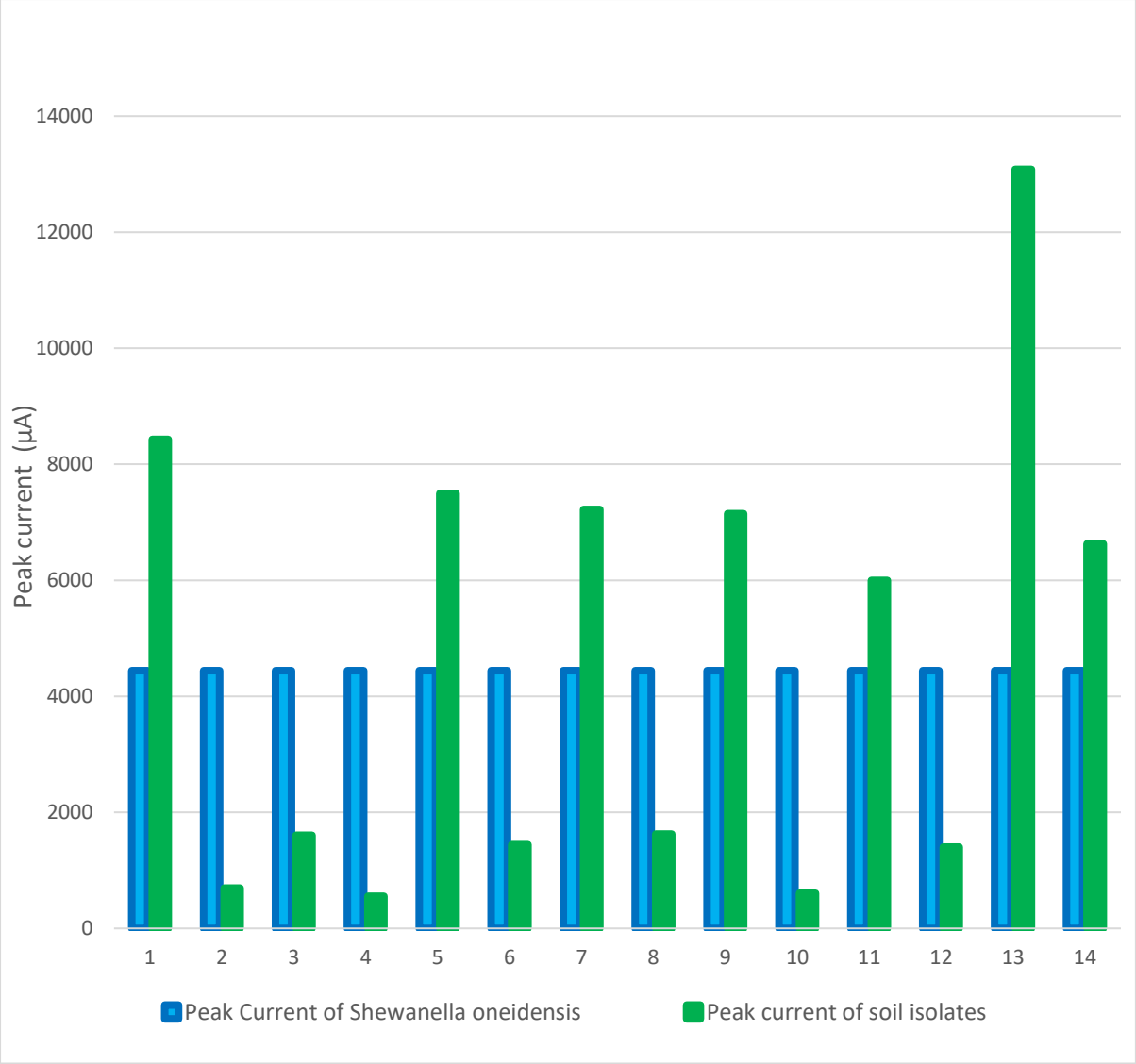


Figure 3.13 Electrochemical activity of microorganisms isolated from petroleum hydrocaron polluted soil compared with a positive control (Shewanella oneidensis). Peak current (µA) and potential (V) were carried out at a scan rate of 10 mV/s.

From **Figure 3.13** shows that the peak current varied from one isolate to the other indicating different electrochemical activity capacity for each. 50% of the total number of the soil isolates (7 isolates out of 14) had a peak current that was greater than S. oneidensis. These 7 isolates had peak current percentages that were greater than that of S. oneidensis ranging from 35% to 194% while the percentage of the other 7 whose peak current were less than *S. oneidensis*'s ranged between -88% to -63%. From this result, it can be seen that the soil isolates had an

overall good electrochemical activity when compared to S. oneidensis which is an indication of a possible positive outcome if the organisms were used for MFCs experiment

3.5.3.2 Groundwater

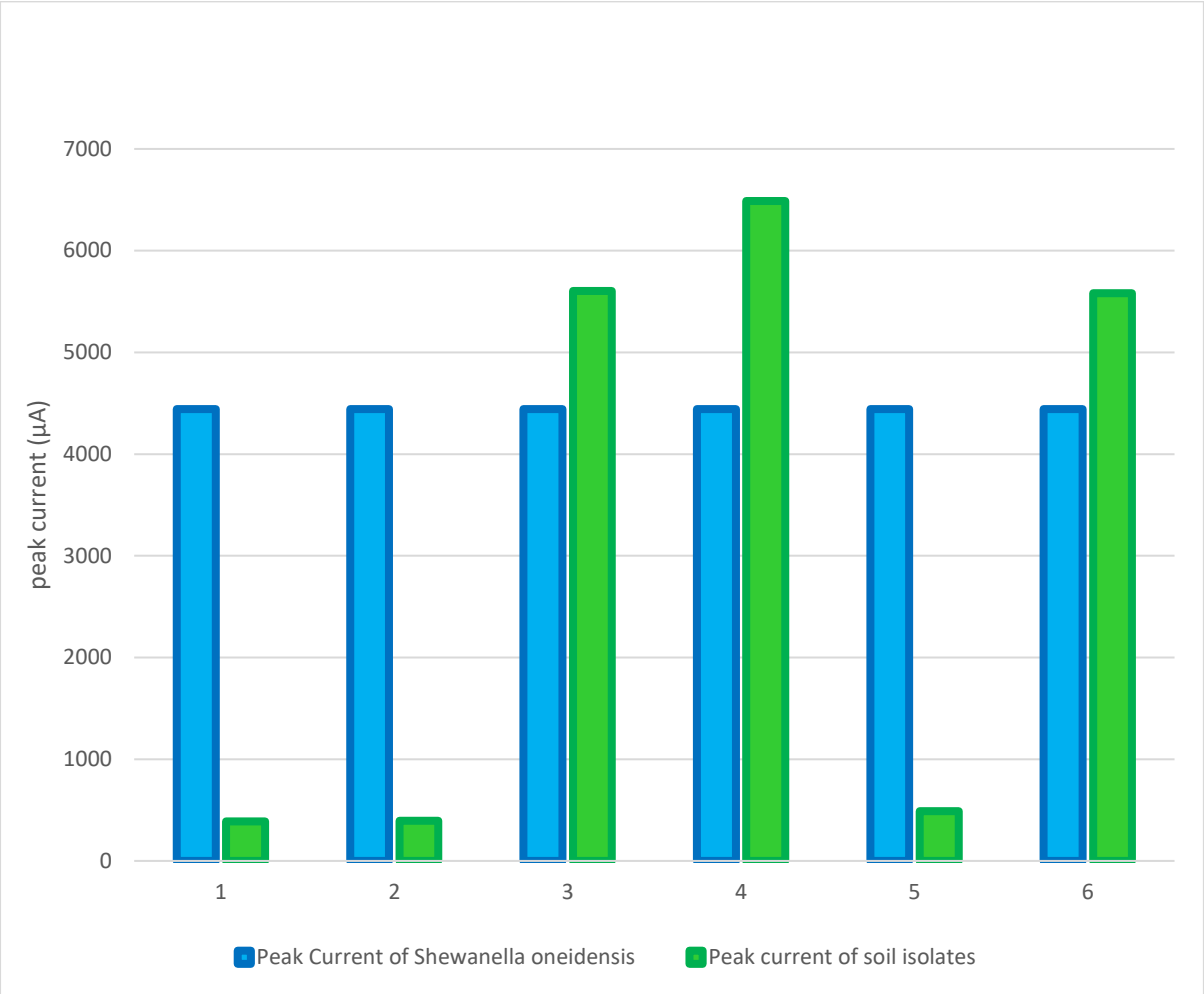


Figure 3.14 Electrochemical activity of microorganisms isolated from petroleum hydrocaron polluted groundwater compared with a positive control (Shewanella oneidensis). Peak current (µA) and potential (V) were carried out at a scan rate of 10 mV/s.

From **Figure 3.14** the petroleum hydrocarbon polluted groundwater isolates peak current varied from one isolate to the other indicating their different electrochemical activity capacity. 50% of the total number of the isolates (3 isolates out of 6) had a peak current that was greater than S. oneidensis. These 3 isolates were greater in peak current percentage ranging from 26% to 46% to that of S. oneidensis while the percentage of the other 3 whose peak current were less than *S. oneidensis's* ranged from -91% to 89%. From this result, it can be seen that the

groundwater isolates had an overall average performance less than the electrochemical activity *S. oneidensis*. However, this is not considered as a limitation as these isolates had recorded good electrochemical activity in an ealier experiment with a negative control. The lower peak current might be as a result of visible thick biofilms that were observed which may have served as insulations to sustrates fast flow and uptake by the organisms directly on the surface of the electrode thereby, affecting the electron transfers to the electrode. Moreover, biosufactant production was also one criteria for selection.

3.5.3.3 Sediment

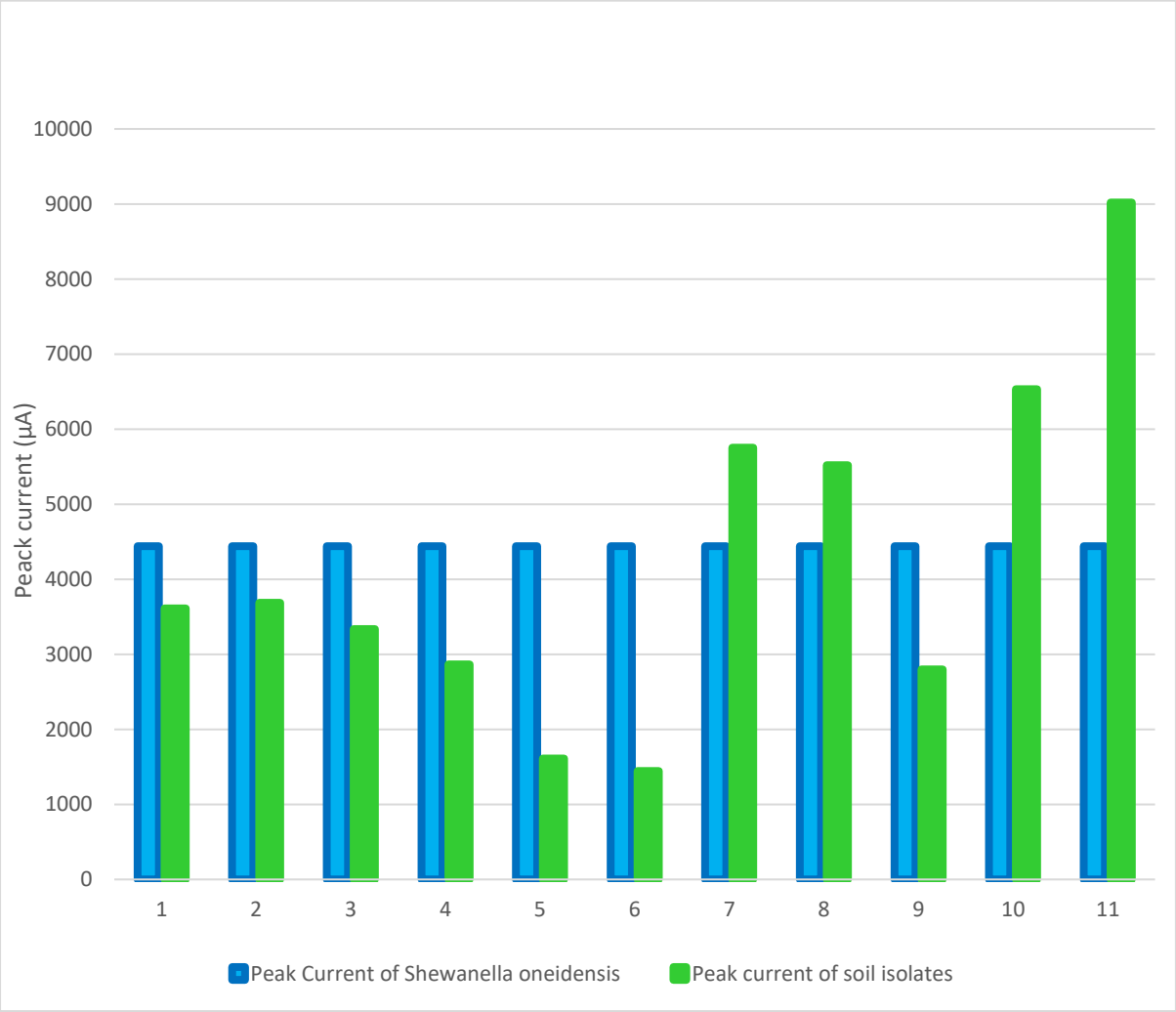


Figure 3.15 Electrochemical activity of microorganisms isolated from petroleum hydrocaron polluted sediment compared with a positive control (Shewanella oneidensis). Peak current (µA) and potential (V) were carried out at a scan rate of 10 mV/s.

From **Figure 3.15** the petroleum hydrocarbon polluted sediment isolates peak current varied from one isolate to the other indicating their different electrochemical activity capacity. 36% of the total number of the isolates (4 isolates out of 11) had a peak current that was greater than *S. oneidensis*. These 4 isolates were greater in peak current percentage ranging from 24% to 103% to that of *S. oneidensis* while the percentage of the other 3 whose peak current were less than S. oneidensis's ranged from -67% to -25%. From this result, it can be seen that the sediment isolates had an overall average performance than the electrochemical activity *S. oneidensis*. However, this is not considered as a limitation as these isolates had recorded good electrochemical activity in an ealier experiment against the negative. The lower peak current might be as a result of visible thick biofilms that were observed which may have served as insulations to sustrates fast flow and uptake by the organisms directly on the surface of the electrode thereby, affecting the electron transfers to the electrode. Moreover, biosufactant production was also one criteria for selection.

From the results, the isolates from the different matrices exhibited a distinctive electrochemical activity. When compared to both the negative and positive, a significant numer exhibited excellent to good electrochemical activity. Those that had 100% or higher to the negative control are selected to form the members of the microcosm for each matrix. Soil had the highest with 14 isolates followed by sediment with 11 and groundwater 6.

3.6 Biosurfactants

3.6.1 Overview

Surfactants are groups of chemical compounds that are known as amphiphilic compounds that possess both hydrophilic and hydrophobic regions. These compounds have become an integral part of many sectors of modern industry with significant roles in industry such as bioremediation, food and beverage, agriculture, healthcare/medicine and textiles. Most of

the surfactant being used are synthesized through organo-chemical synthesis and petrochemicals as precursors. This has resulted in efforts towards discovering of surfactants that are from biological sources known as biosurfactants in recent times (Nikolova and Gutierrez., 2021). With the current efforts, the global market of biosurfactant was estimated to be 4.20 billion dollars in 2017 and has a projected growth of about 5.52 billion dollars in 2022. The potential advantages of biosurfactant over chemical surfactants is their Eco-friendliness that include biodegradability, high selectivity, bioavailability, biocompatibility, ecological acceptability, increased effectiveness in high temperature and salt concentrations (Ambaye et al., 2021). Different microorganisms are said to produce different types of biosurfactants. While the biosurfactants produced by Bacillus sp. are reported to be lipopeptide in nature such as surfactin, lichenysin, iturin, and fengycin (Felix et al., 2019; Chen et al., 2017; Li et al., 2008) those produced by Pseudomonas sp. are reported to be glycolipid in nature such as rhamnolipids (Kaskatepe and Yildiz, 2016). Biosurfactants such as glycolipid and Trehalose have been reported as enhancing faster biodegradation of petroleum hydrocarbon by solubilising the spilled oil and making them available to hydrocarbon-degraders (Souza et al., 2014). In another study of petroleum hydrocarbon remediation, rhamnolipids and sophorolipids solubilised petroleum hydrocarbon pollutants into aqueous phase thereby increasing their bioavailability for biodegradation (Aulwar and Awasthi., 2016).

3.6.2 Sediment

Three of the isolates from the petroleum hydrocarbon polluted sediment produced biosurfactants that emulsified vegetable oil as shown in **Figure 3.16**. With an oil layer of 2 cm, these isolates that were all highly similar to Pseudomonas sp. had 99%, 70% and 30% emulsification index of the oil layer i.e. the layer of oil emulsified by biosurfactant. This genus has been previously reported by Phulpoto et al. (2021) to produced biosurfactants with an

emulsification index within the range of 32.7-40.60% for after a 4-day incubation period. Although the study by Phulpoto et al. (2021) had a slightly different experimental set up and duration, the emulsification index by the isolates from the petroleum hydrocarbon polluted sediment had a higher emulsification index.

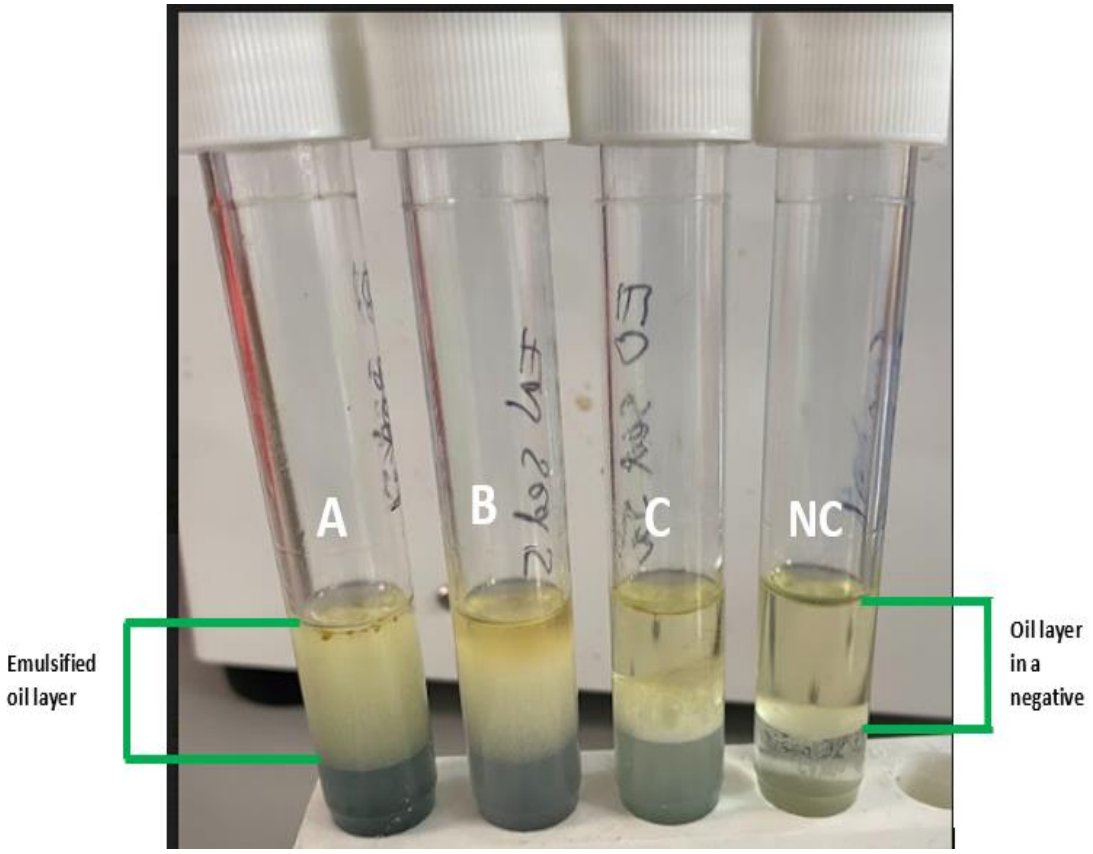


Figure 3.16 A, B and C shows different degree of emulsified layer of vegetable oil by biosurfactant that were produced by bacteria isolated from petroleum hydrocarbon polluted sediment while NC is the negative control.

3.6.3 Groundwater

An isolate from the petroleum hydrocarbon polluted groundwater produced biosurfactants that emulsified vegetable oil as shown in **Figure 3.17**. With an oil layer of 2 cm, this isolate that was identified by blast to be highly similar to a Pseudomonas sp. had 35% emulsification index. This result is similar to previously reported study by Phulpoto et al. (2021) in which isolates produced biosurfactants with an emulsification index within the range of 32.70-

40.60%. Although the study by Phulpoto et al. (2021) had a different experimental set up and duration, the emulsification index by the isolate from the petroleum hydrocarbon polluted groundwater had a similar emulsification index.

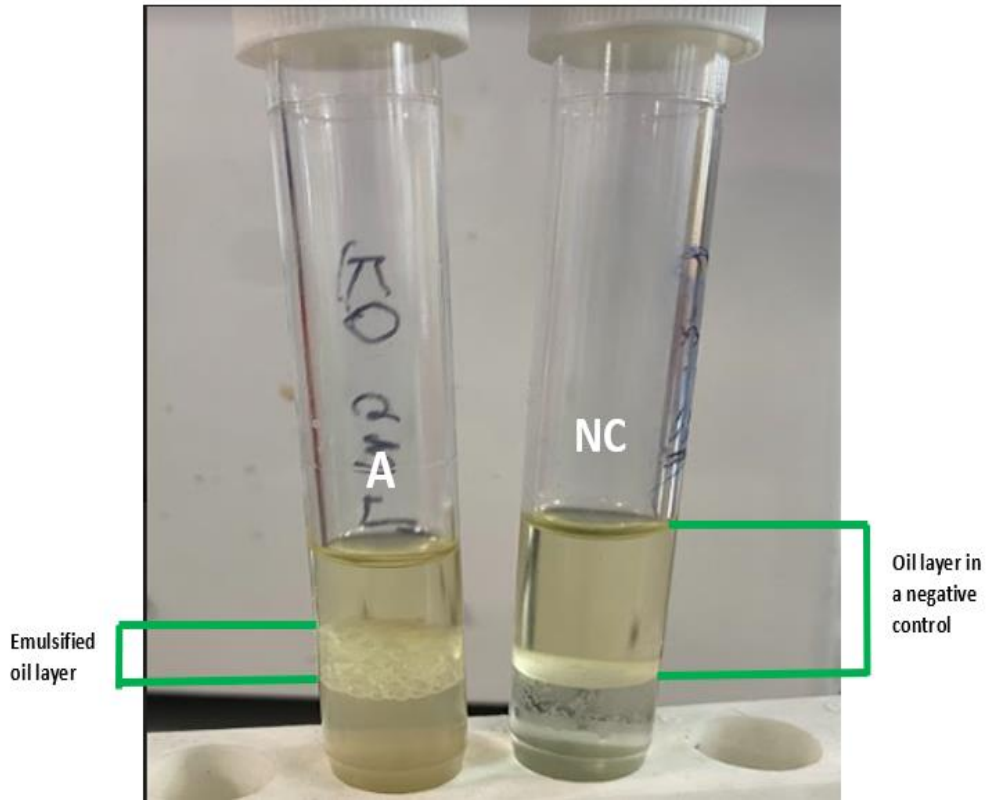


Figure 3.17 A shows an emulsified layer of vegetable oil by biosurfactant that was produced by a bacterium isolated from petroleum hydrocarbon polluted groundwater while NC is the negative control.

3.6.4 Soil

Two isolates from the petroleum hydrocarbon polluted soil produced biosurfactants that emulsified vegetable oil as shown in **Figure 3.17**. With an oil layer of 2 cm, this isolate that were identified by blast to be highly similar to a Pseudomonas sp. had 35% and 45% emulsification index. This result is similar to previously reported study by (Phulpoto et al., 2021) in which isolates produced biosurfactants with an emulsification index within the range of 32.70-40.60%. Although the study by Phulpoto et al. (2021) had a different experimental

set up and duration, the emulsification index by these isolates from the petroleum hydrocarbon polluted soil was within a similar emulsification index.

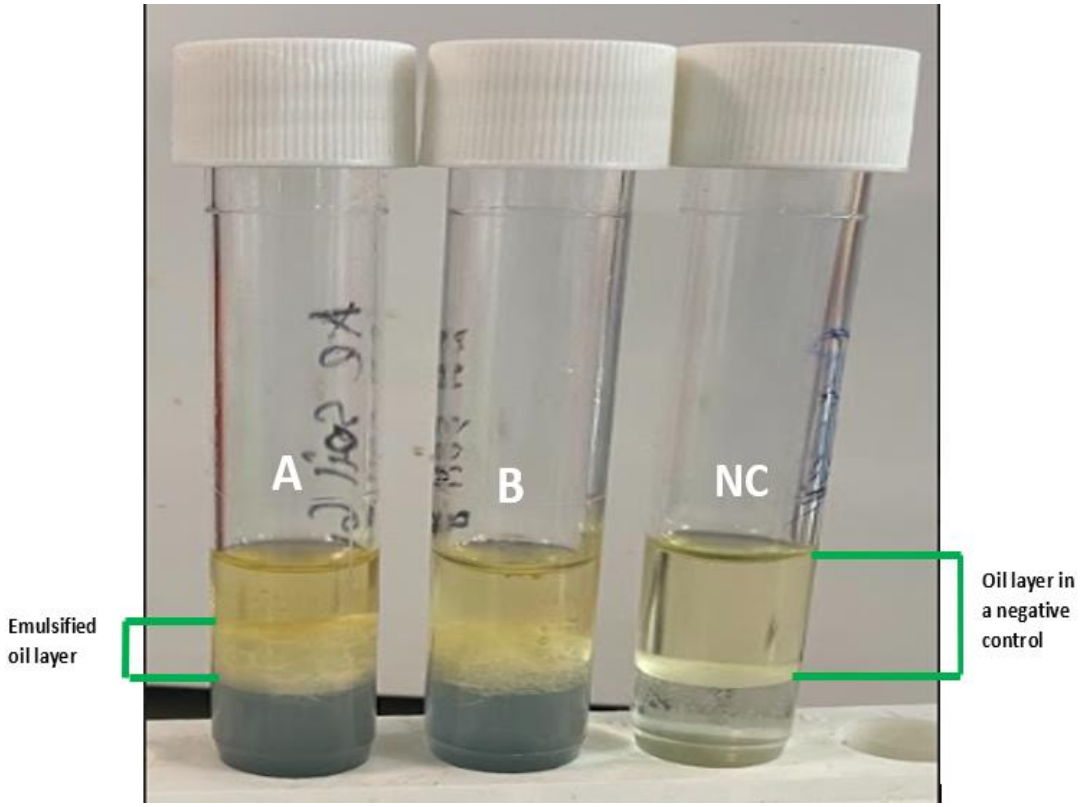


Figure 3.18 A and B shows different degree of emulsified layer of vegetable oil by biosurfactant that was produced by bacteria isolated from petroleum hydrocarbon polluted soil while NC is the negative control.

3.7 Conclusion

The two culture-based approaches, screening pressures (anaerobic growth condition, PH as the sole carbon source and Iron III oxide and manganese IV oxide as electron acceptors) were used to isolate 19, 16 and 9 morphologically distinctive bacteria from PH polluted soil, sediment and groundwater respectively of the Niger-Delta. Using 16s rRNA region, 88% and 84% isolates from groundwater and soil were identified to be highly related to species belonging to the phylum Proteobacteria while those from sediment were 44% each of Proteobacteria and Firmicutes. In the metagenomic analysis, Proteobacteria was the

predominant phylum with 59.3% for groundwater and 26% for soil while sediment had Campilobacterota 19% as the dominant phylum. Generally, these results corroborate with previous studies that reported Proteobacteria as the dominant phylum in PH polluted matrices and some of its species like Pseudomonas sp. as good PH degraders.

Biosurfactant production by microorganisms enhances the remediation of PH pollutants by solubilising them into aqueous phase that increases their bioavailability for degradation. 3, 2 and 1 isolates from sediment, soil and groundwater produced biosurfactant. The Emulsification Index (EI) for isolates were sediment 99%, 70% and 30%, soil 45% and 35% and groundwater 35%. The EI of 99%, 70% and 45% by these isolates is above the 32.7-40.6% reported in previous studies.

The biocatalysts for BES are electro active microorganisms. For this, the electrochemical activity of each isolate was assessed. 11 (87.5%), 14 (84.2%) and (6) 77.78% of isolates for sediment, soil and groundwater respectively generated peak currents that were greater than that of the negative control. Isolates that exhibited good electro activity with a peak that is 100% greater than that of the negative control and those that produced biosurfactants were selected to be integrated into MFC experiments. The first phase of the MFCs experiment used benzene as the sole source of carbon while the second phase used co-substrate of benzene and phenanthrene.

Chapter 4: Comparing microbial fuel cell outputs of pure strains and microcosm

4.1 Overview

The microbial community plays a significant role in biochemical cycles, biodegradation and chemicals production (Islam et al., 2018; Wang et al., 2015). Due to their versatility, these microorganisms have been reported to degrade a wide range of organic compounds including petroleum hydrocarbons in bioelectrochemical systems. The efficiency of bioelectrochemical remediation of petroleum hydrocarbons is dependent on the syntrophic and synergistic relationship that exist between the members of the community (Aulenta et. al, 2021), these relationships have also been reported to define the evolutionary fate of the microbial consortia in regard to the dynamics and stability of the system (Logan et al., 2019). While there are diverse interactions between microorganisms such as synergism, antagonism, neutralism, amensalism and commensalism, etc. (Zhang et al., 2014; Zhang et al., 2018), synergism that involves the exchange of chemical compounds between microorganisms has been reported to have a positive influence leading to a higher collective output of the consortia compared to the individual performances of each species (D'Souza et al., 2018; Wang et al., 2014). In this chapter, the pure colony's MFCs outputs are compared with the microcosm's MFCs outputs of the screened and selected microorganisms with the desirable characteristics as recorded in Chapter 3. Although the electricity generation can be considered a bonus to research aimed at remediation of environmental pollutants, the voltage output serves as a real-time indicator for measuring the level of the degradation activity being carried out by the microorganisms (Wang et. al., 2020c). The analysis of the several outputs in this chapter formed the bases of either using a pure colony from each matrix or a combination of all the pure colonies from each matrix to form a microcosm for that matrix in the succeeding experiment is most suitable. The MFCs outputs for the pure colony and microcosm for each matrix are presented and analysed below.

4.2 Voltage output of pure strains vs microcosm

4.2.1 Groundwater

In **Figure 4.1,** the groundwater microcosms MFCs setup highest voltage was 285.9155 (mV) while the pure colony highest voltage was 35.475 (mV) within the study period of over 160 hours (7 days). The highest microcosm voltage was 706% higher than the highest voltage of the pure colony. In addition to the significant difference in the voltage output between the microcosm and the pure colony, there was also a significant difference between the time that each set up was able to attend the highest voltage. While the microcosm's highest voltage was attained in less than 5 hours of data recording, it took about 140 hours for the pure colony to attain the highest voltage. Owing to the fact that the voltage output serves as a real- time indicator for degradation activities (Wang et. al., 2020c), the above results show that it took a shorter time for the microcosm to have its highest degradation activity compared to the pure strain isolated from the petroleum polluted groundwater in an MFC experiment.

4.2.2 Soil

In **Figure 4.2,** the soil microcosms MFCs setup highest voltage was 279.409 (mV) while the pure colony highest voltage was 19.825 (mV) within the study period of over 160 hours (7 days). The highest microcosm voltage was 1309% higher than the highest voltage of the pure colony. In addition to the significant difference in the voltage output between the microcosm and the pure colony, there was also a significant difference between the time that each set up was able to attend the highest voltage. While the microcosm's highest voltage was attained in less than 5 hours of data recording, it took about 140 hours for the pure colony to

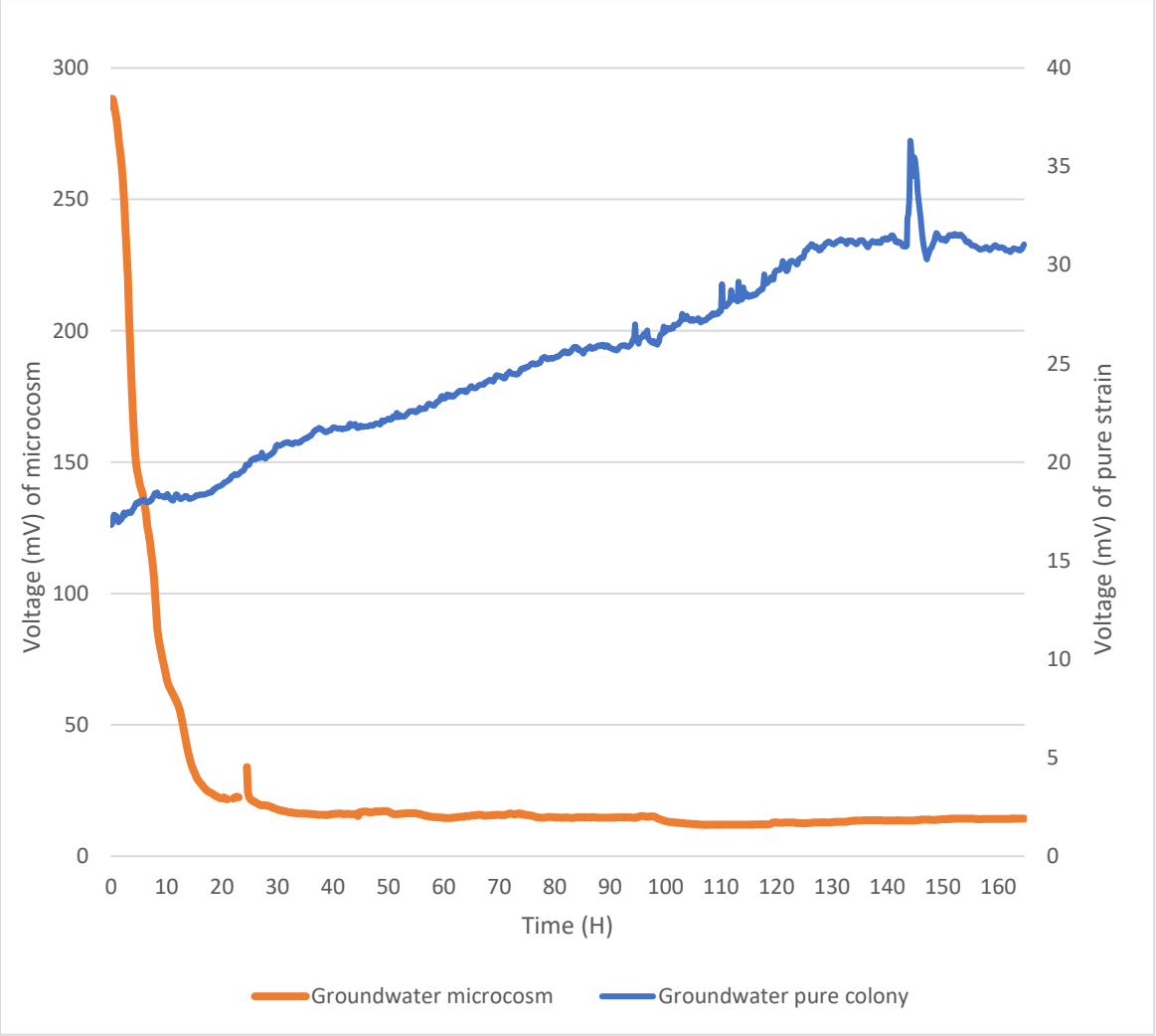


Figure 4.1 Voltage (mV) output from H-type MFCs with benzene as the sole source of carbon and inoculated with petroleum hydrocarbon polluted groundwater microcosms versus a selected pure colony that formed part of the microcosm.

attain the highest voltage. Because the voltage output serves as a real- time indicator for degradation activities (Wang et. al., 2020c), the above results shows that it took a shorter time for the microcosm to have its highest degradation activity compared to the pure strain isolated from the petroleum polluted groundwater in an MFC experiment.

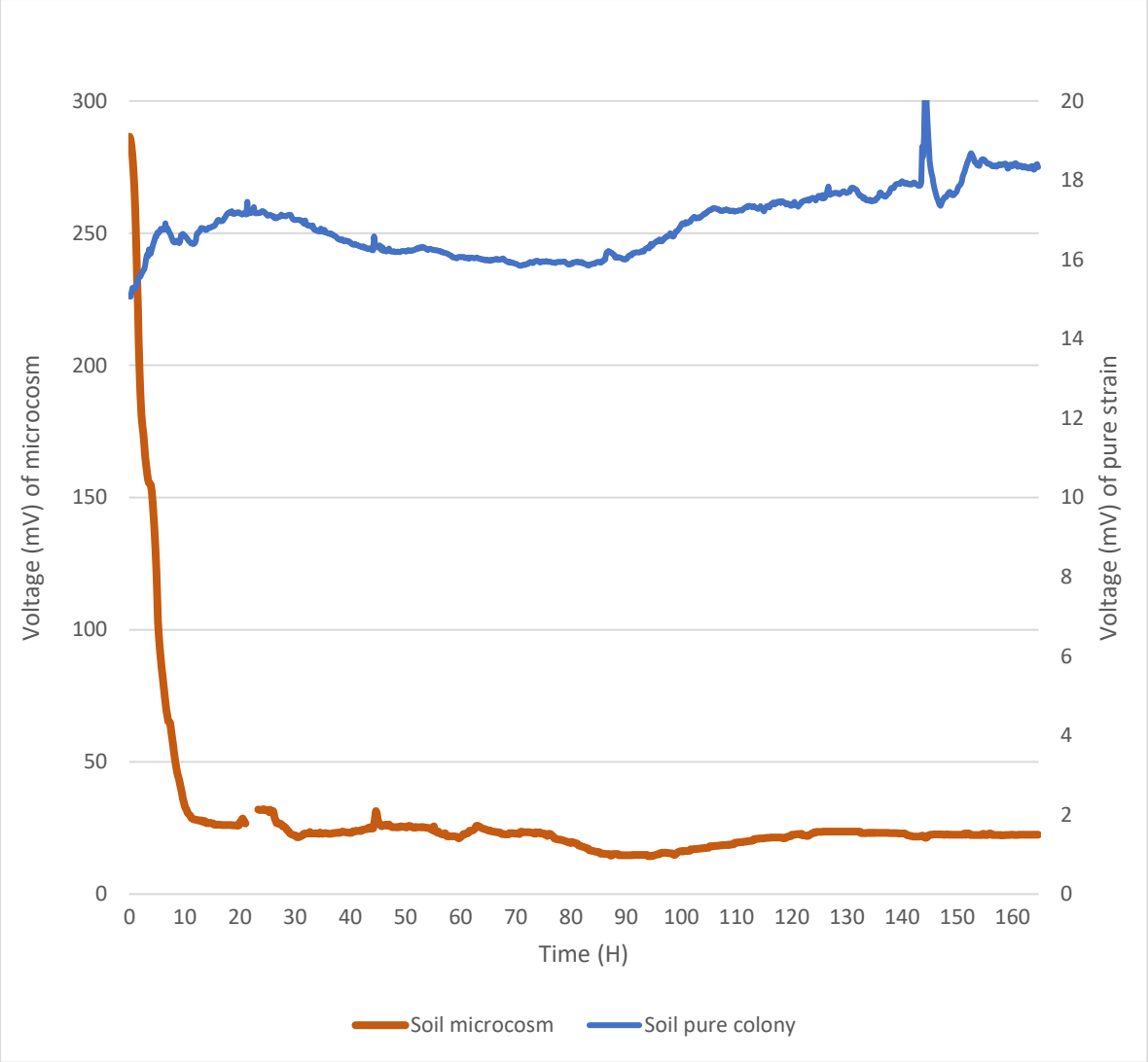


Figure 4. 2 Voltage (mV) output from MFCs with benzene as the sole source of carbon and inoculated with petroleum hydrocarbon polluted soil microcosms versus a selected pure colony that formed part of the microcosm.

4.2.3 Sediment

In **Figure 4.3,** the sediment microcosms MFCs setup highest voltage was 270 (mV) while the pure colony highest voltage was 24 (mV) within the study period of over 160 hours (7 days). The highest microcosm voltage was 1044% higher than the highest voltage of the pure colony. In addition to the significant difference in the voltage output between the microcosm and the pure colony, there was also a significant difference between the time that each set up was able to attend the highest voltage. While the microcosm's highest voltage was attained in less than 5 hours of data recording, it took about 140 hours for the pure colony to attain the

highest voltage. Since the voltage output serves as a real- time indicator for degradation activities (Wang et. al., 2020c), the above results shows that it took a shorter time for the microcosm to have its highest degradation activity compared to the pure strain isolated from the petroleum polluted groundwater in an MFC experiment

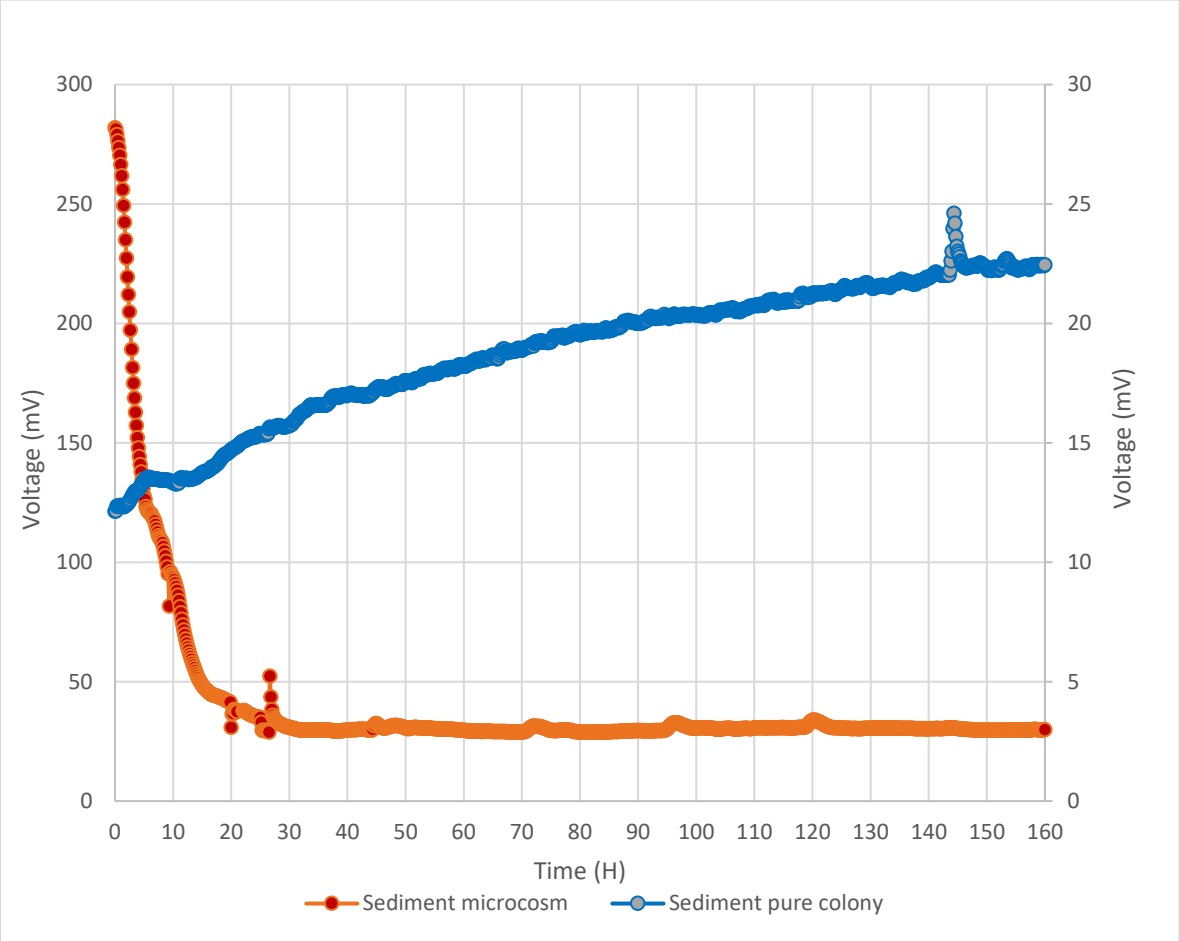


Figure 4.3 Voltage (mV) output from H-type MFCs with benzene as the sole source of carbon and inoculated with petroleum hydrocarbon polluted sediment microcosms versus a selected pure colony that formed part of the microcosm.

The results in **Figures 4.1, 4.2 and 4.3** shows that the microcosm experiments in all the matrices (groundwater, soil and sediment) had a higher voltage output compared to that of the pure colony. This aligns with the synergistic relationship that is said to exist between microorganisms that involves the exchange of chemical compounds between them, thereby resulting in a positive influence that leads to a higher collective output of the consortia (microcosm) compared to the individual performances of each species (pure colony) (D'Souza

et al., 2018; Wang et al., 2014). Furthermore, the microcosm experiments across the three matrices attained their highest voltage output in less than 5 hours (less than a quatre of a day) from the commencement of recording while the pure colony experiments attained their highest voltage output about 140 hours (more than 5 days). Since the voltage output can serve as the measure of degradation activity, this implies that the microcosm experiment had the highest degradation activity and at the fastest time.

4.3 Power density and polarisation curve of microcosm vs pure strain in MFCs

4.3.1 Sediment

Comparing the MFCs output between the microcosm and the pure colony from the petroleum hydrocarbon polluted sediment in **Figure 4.4,** there is a clear distinction in both the polarisation curve and the power density for the microcosm and pure colony labelled A and B respectively. While the highest power density for the microcosm was 498 (uW/m^2) that of the pure colony was 53 (uW/m^2). From this result, the power density for the microcosm was 843% greater than that of the pure colony. The polarisation curve of both the microcosm and the pure colony showed a significant difference in the peak voltage (mV) at the lowest current density (mA/m^2)**.** The voltage for the microcosm was 340 (mV) while that of the pure colony was 45.6 (mV) making the voltage of the microcosm 645.614% greater than that of the pure colony.

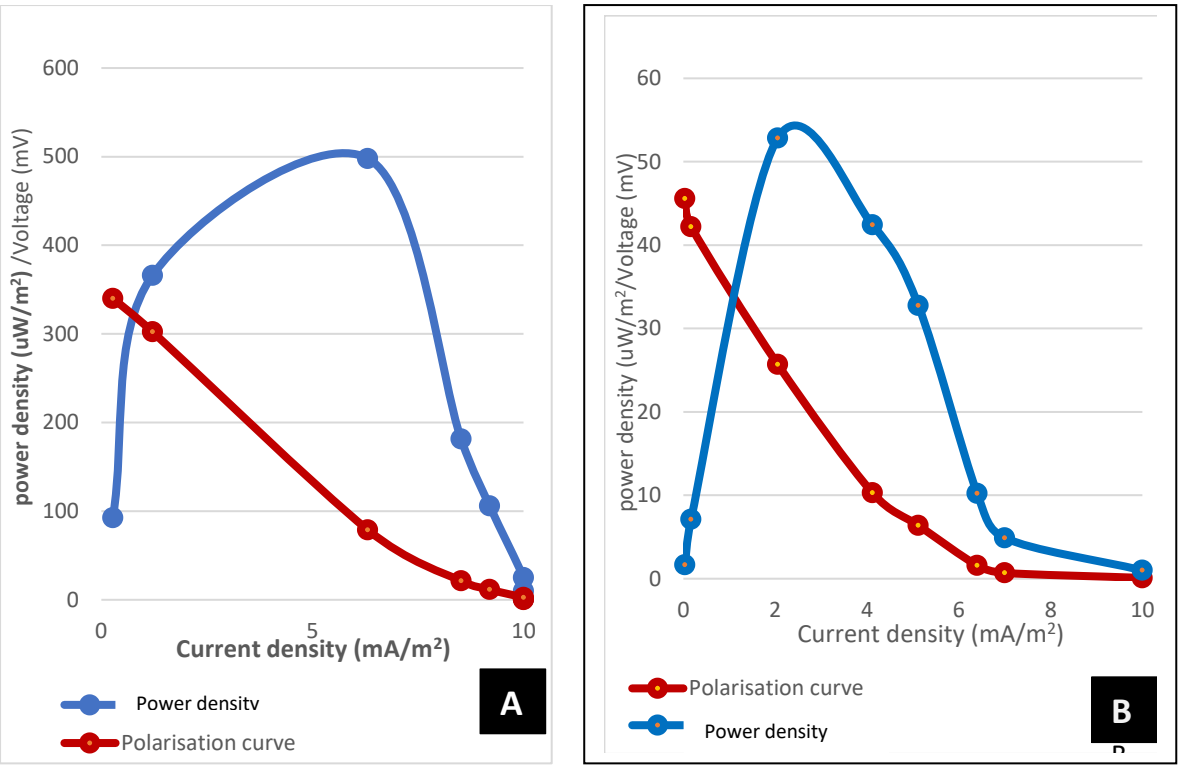


Figure 4.4 Polarisation curve (Peak voltage (mV)) and power density (uW/m^2) outputs from H-type MFC with benzene as the sole source of carbon and inoculated with microcosm and pure strain MFC labelled A and B respectively isolated from petroleum hydrocarbon sediment.

4.3.2 Soil

Comparing the MFCs output between the microcosm and the pure colony from the petroleum hydrocarbon polluted soil in **Figure 4.5,** there is a clear distinction in both the polarisation curve and the power density for the microcosm and pure colony labelled A and B respectively. While the highest power density for the microcosm was 362 (uW/m^2) that of the pure colony was 76 (uW/m^2). From this result, the power density for the microcosm is 374.3656% greater than that of the pure colony. The polarisation curve of both the microcosm and the pure colony showed a significant difference in the peak voltage (mV) at the lowest current density (mA/m^2)**.** The voltage for the microcosm was 307.7 (mV) while that of the pure colony was 91 (mV) making the microcosm's voltage 238% greater than that of the pure colony.

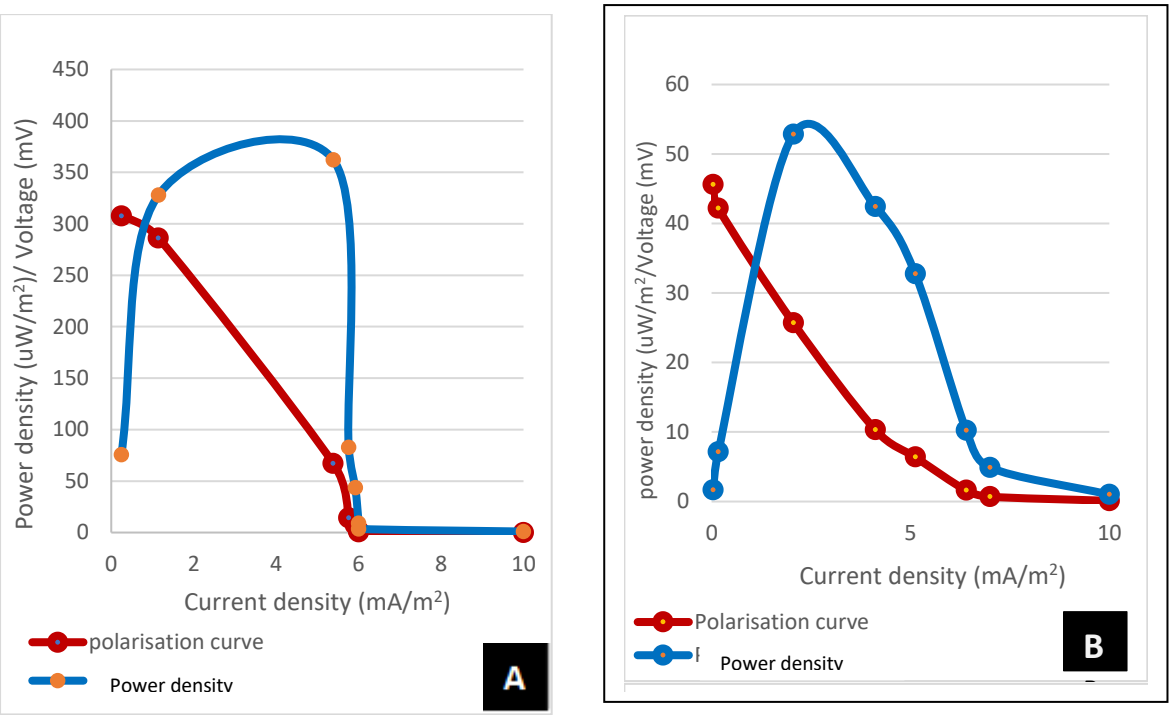


Figure 4.5 Polarisation curve (Peak voltage (mV)) and power density (uW/m²) outputs from H-type MFC with benzene as the sole source of carbon inoculated with microcosm and pure strain MFC labelled A and B respectively isolated from petroleum hydrocarbon soil.

4.3.3 Groundwater

Comparing the MFCs output between the microcosm and the pure colony from the petroleum hydrocarbon polluted groundwater in **Figure 4.6,** there is a clear distinction in both the polarisation curve and the power density for the microcosm and pure colony labelled A and B respectively. While the highest power density for the microcosm was 222 (uW/m²) that of the pure colony was 57 (uW/m²). From this result, the power density for the microcosm is 293% greater than that of the pure colony. The polarisation curve of both the microcosm and the pure colony showed a significant difference in the peak voltage (mV) at the lowest current density (mA/m²)**.** The voltage for the microcosm was 295 (mV) while that of the pure colony was 26.6 (mV) making the microcosm's voltage 1007% greater than that of the pure colony. Comparing the results, there is a similar trend across the matrices and the outputs differences between the microcosm and the pure colony MFCs. Generally, the microcosm set up had

higher power density and peak voltage compared to the pure strain set up in **Figure 4.4, 4.5** and **4.6**.

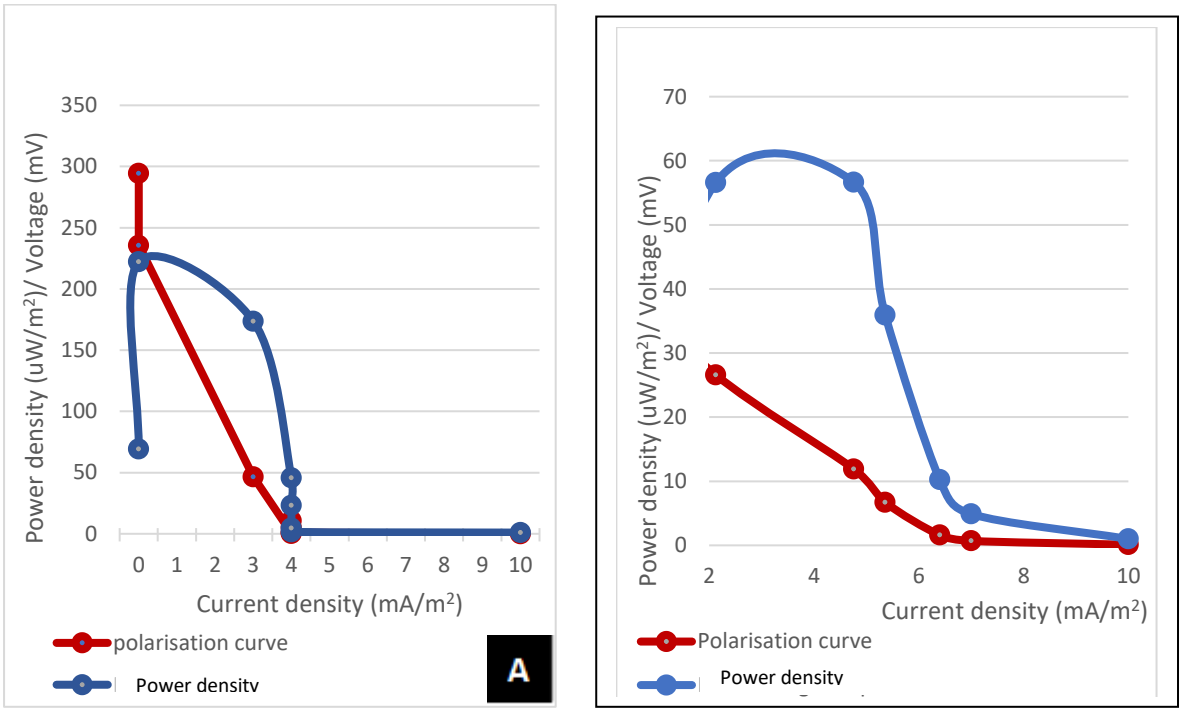


Figure 4.6 Polarisation curve (Peak voltage (mV)) and power density (uW/m^2) outputs from H-type MFC with benzene as the sole source of carbon and inoculated with microcosm and pure strains MFC labelled A and B respectively isolated from petroleum hydrocarbon groundwater.

4.4 Conclusion

From the overall results, there is a similar trend of differences between the microcosm MFCs outputs and the pure colony MFCs across the isolate from the three matrices. The microcosm experiments had the highest power density and voltage compared to the pure colony in all the three matrices. Although these microbes were isolated from different sources, the output from the experiments shows that a combination of selected microcosms with desired characteristics resulted in better outputs than using a pure colony with the same characteristics. This better output is indeed a reflection of the synergistic relationships that exist between different microbial species in a community. These outcomes align with previous

studies (D'Souza et al., 2018; Wang et al., 2014) that describe synergism as a relationship that involves the exchange of chemical compounds between microorganisms leading to a higher collective output of the consortia compared to the individual performances of each species. From the MFCs outputs in this chapter, the data showed that the use of microcosm in MFCs experiments have better outputs compare to the use of a single species (pure strain). Therefore, going forward, the microcosms were used in all subsequent experiments. Microcosm formulated from each pf the matrix was used solely in a laboratory experiment that closely mimic the petroleum polluted matrix that the microorganisms were isolated from as recorded in Chapter 3. The three microcosms formulated from the petroleum polluted soil, sediment and groundwater were used in experiments that closely mimic soil, sediment and groundwater respectively. Therefore, all subsequent experiments were independent of one another and were significantly different from the microcosm used, matrix design, MFCs configuration and bioreactor.

Chapter 5: Comparing the effects of chemical surfactants and biosurfactants on phenanthrene biodegradation in microbial fuel cells

5.1 Overview

The widespread of environmental pollution by petroleum hydrocarbon has been reported in different part of the world. Groundwater is one of the matrices that these pollutants affect. Owing to the toxicity of these pollutants to the environment and human health, there have been several approaches to remediate these pollutants from the environment. More details on these can be found in Chapter 1 above. One of the major challenges of remediating these pollutants is their hydrophobic nature resulting to low solubility in water leading to the formation of Non-Aqueous Phase Liquids (NAPL) that absorb strongly to environmental matrices. These have characteristics have resulted to a rebound and tailing effects that are often observed during the remediation of these pollutants in the environment. This has made achieving remediation goals difficult as the concentration decline during the remediation period is often short lived by a rise in the concentration of the contaminant after the remediation has been completed. This rise in concentration of pollutants can be said to be attributed to the pollutants' molecules that absorb to the polluted matrix (O'Connor et al., 2018) and the non-bioavailability of these pollutants for biodegradation (Adelaja, 2015). These have necessitated to the wide application of surfactants to enhance the remediation of petroleum hydrocarbon polluted matrices in other to enhance the process (Davin et al., 2018; Pei et al., 2017; Alcantara et al., 2009; Lai et al., 2009). Surfactants are said to play a key role in facilitating the desorption of solid phase petroleum hydrocarbon pollutants and enhancing the dissolution of nonaqueous phase liquids by reducing the oil/water interfacial tension and the air/water surface tension of solution (Wei et al., 2020; Zhou et al., 2011). The results below are based on the outcome of groundwater MFC experiment inoculated with the microcosm formulated from pure strains isolated from the polluted petroleum hydrocarbons groundwater Chapter 3, supplemented with biosurfactant and chemical surfactants.

5.2 Voltage output of groundwater MFC

From the result shown in **Figure 5.1** all the three experiments produced a similar pattern for the voltage output. They all produced the highest voltage at the beginning of the experiment that gradually declined and began to stabilise after the 40th hour. While those inoculated with the microcosm alone and one supplemented with biosurfactant produced an almost steady voltage to the end after declining. It can also be seen that the MFC supplemented with Tween 80 had a slightly voltage spike and remained averagely steady to the end. In the overall, there is a similar trend with the voltage of the groundwater microcosm in Figure 4.1. However, there is a difference in the peak voltage recorded in both. As microbes are said to degrade shorter and less complex petroleum hydrocarbons relatively more easily (Van Hamme et al., 2003), the difference in the peak voltage can be attributed to the different type of substrates used as benzene was the sole source of carbon in Figure 4.1, while in Figure 5.1 a co-substrate of benzene and phenanthrene was used. When the peak voltage in the three experimental setups in Figure 5.1 are compared, there is no significant difference as they all were constituted of same microcosm and substrate. It appears that the surfactant supplement was yet to have a significant effect that could result in significant difference in the voltage outputs of the three set ups.

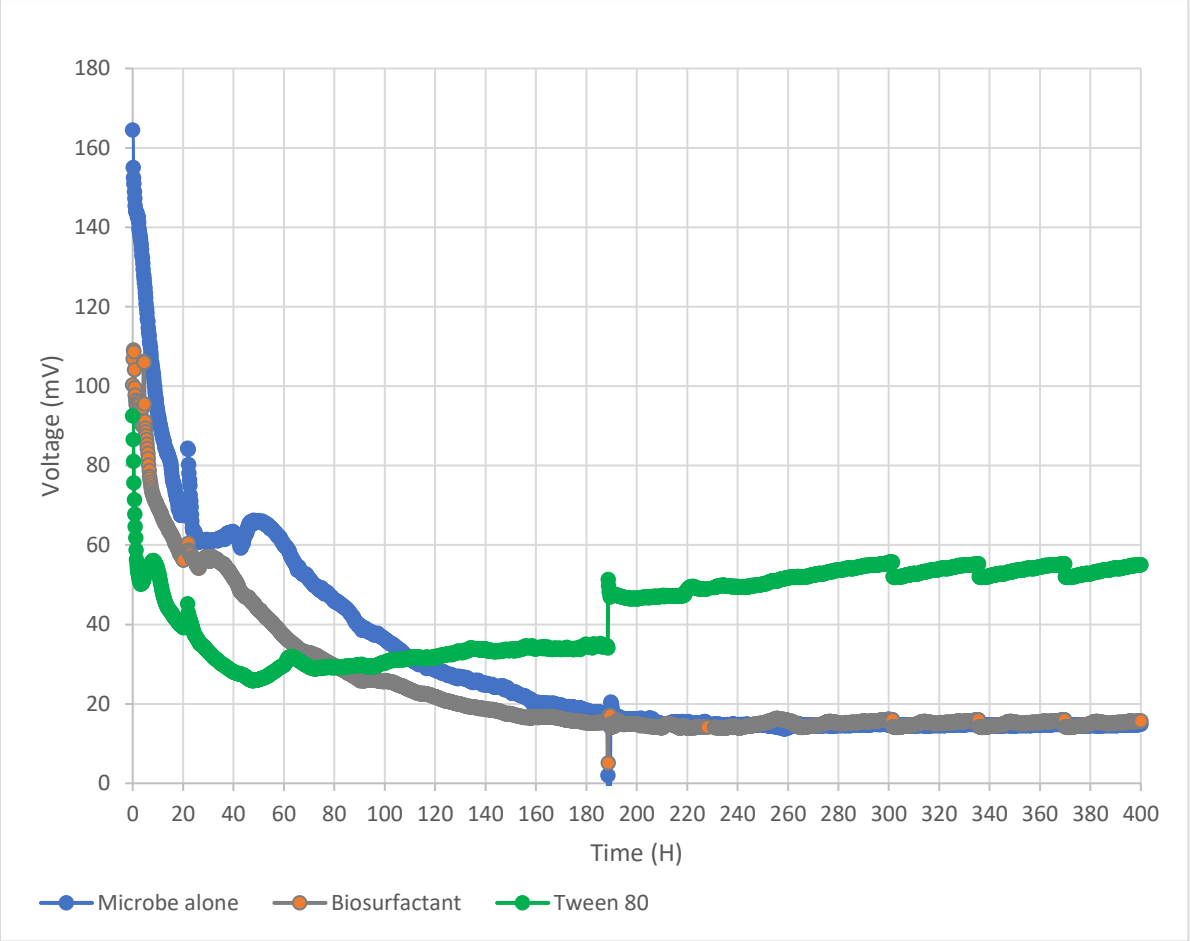


Figure 5.1 Voltage (mV) output over time from H-type MFCs inoculated with petroleum hydrocarbon polluted groundwater microcosms with benzene and phenanthrene as co-substrate in three different experimental set-ups: Microcosms alone, the microcosms supplemented with biosurfactant and the microcosms supplemented with chemical surfactant (Tween 80).

5.3 Polarisation curve of groundwater MFC

Although there were varying voltages for the polarisation curve in **Figure 5.2,** it can be seen that the three inoculated with the microcosm alone and those supplemented with surfactant had similar trend which in turn implies similar working condition and similar internal resistance. However, when compared with the polarisation curve of the microcosm in Figure 4.5 there is significant difference in both the trend and the peak voltage recorded which simply indicates a difference system. It is important to note that the microcosms, electrode, temperature and other variables used are the same in both Figure 4.5 and Figure 5.2 except

for the substrate that is different. While benzene was the sole source of carbon in Figure 4.5, in Figure 5.2 it was a co-substrate of benzene and phenanthrene these could be responsible for the significant difference as the type of substrate used in an MFC can influence the entire system and the difference be reflected in a polarisation curve.

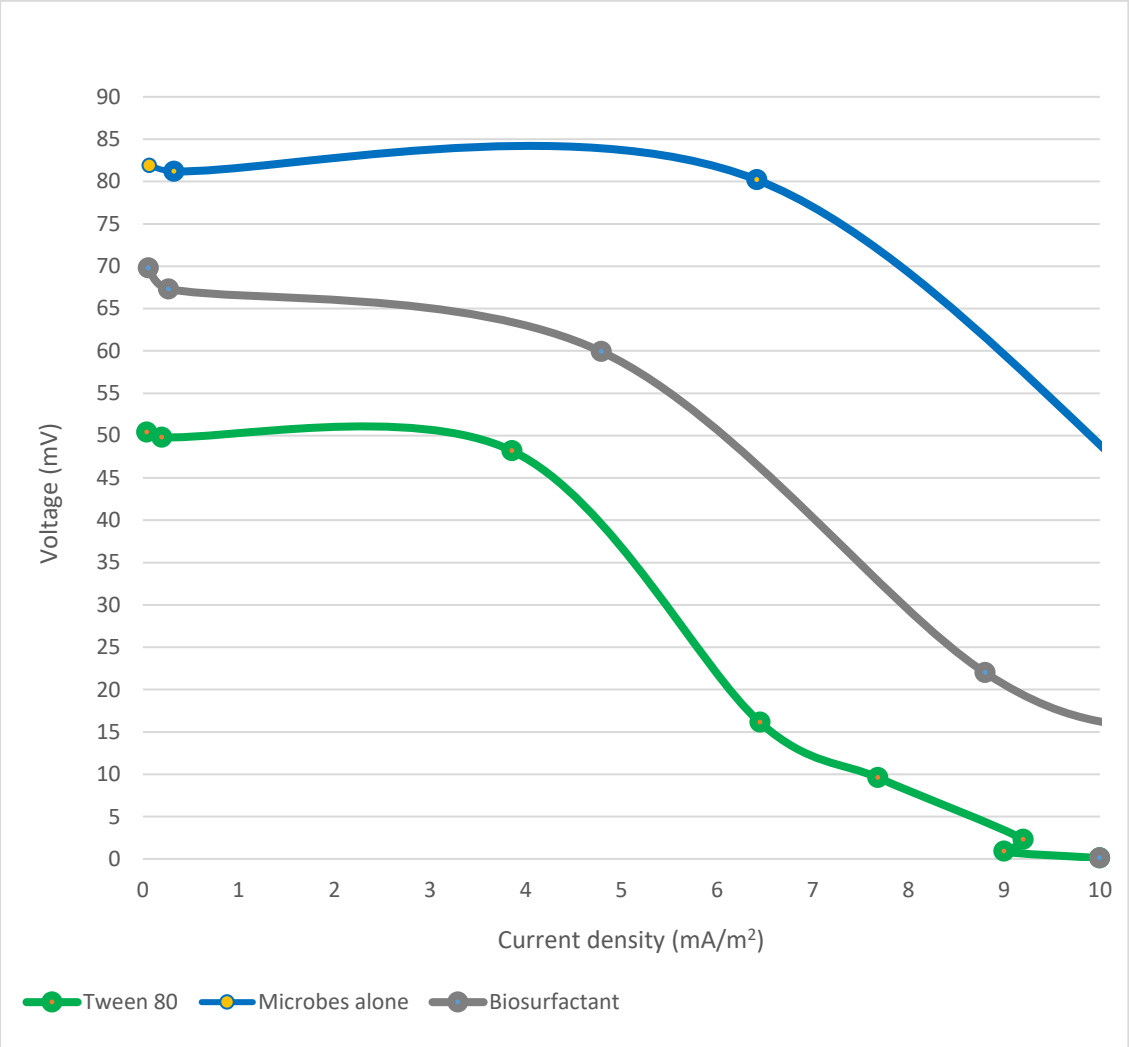


Figure 5.2 Polarisation curve from a H-type MFCs inoculated with petroleum hydrocarbon polluted groundwater microcosms with benzene and phenanthrene as co-substrate in three different experimental setups: Microcosms alone, the microcosms supplemented with biosurfactant and the microcosms supplemented with chemical surfactant (Tween 80).

5.4 Power density of groundwater MFC

From **Figure 5.3** showed that the power density for the three set ups were significantly different from each other. The highest power density 515 (uW/m^2) was recorded for the set

up that had only the groundwater microcosm without any surfactant supplement. The setup supplemented with biosurfactant had the second highest power density of 287 (uW/m^2) while the setup supplemented with Tween 80 had the least power density of 186 (uW/m^2). The result indicated that supplementing surfactants in an MFC for the degradation of co-substrates of petroleum hydrocarbon can significantly influence the power density output. There have been previous reports that higher power density taken at a given time is not always directly proportional to the degradation of the substrates (Adelaja, 2015; Hu et al. 2011). However, higher power density was said to be more associated with the presence of readily oxidisable compounds (Adelaja, 2015). Perhaps the highest peak power density recorded for the microcosms alone could be as a result of the absence of a surfactant that could facilitate the dispersion (dissociation) of phenanthrene to increase its availability for degradation making benzene as the almost sole source of carbon. According to Guo et al. (2020) that the presence of an electrode can promote electrochemical oxidation that is capable of enhancing the conversion of high-molecular-weight polycyclic aromatic hydrocarbons to small-molecular weight aromatics or linear-aliphatic hydrocarbon in a system. Perhaps there was significant energy that was utilised to drive the conversion process of the dispersed phenanthrene to smaller-molecular weight petroleum hydrocarbon in the bioreactors that contained surfactants compared to the bioreactor with microbes alone. Thereby leading to less power density for surfactants supplemented MFCs compared to the MFC with the microorganisms alone.

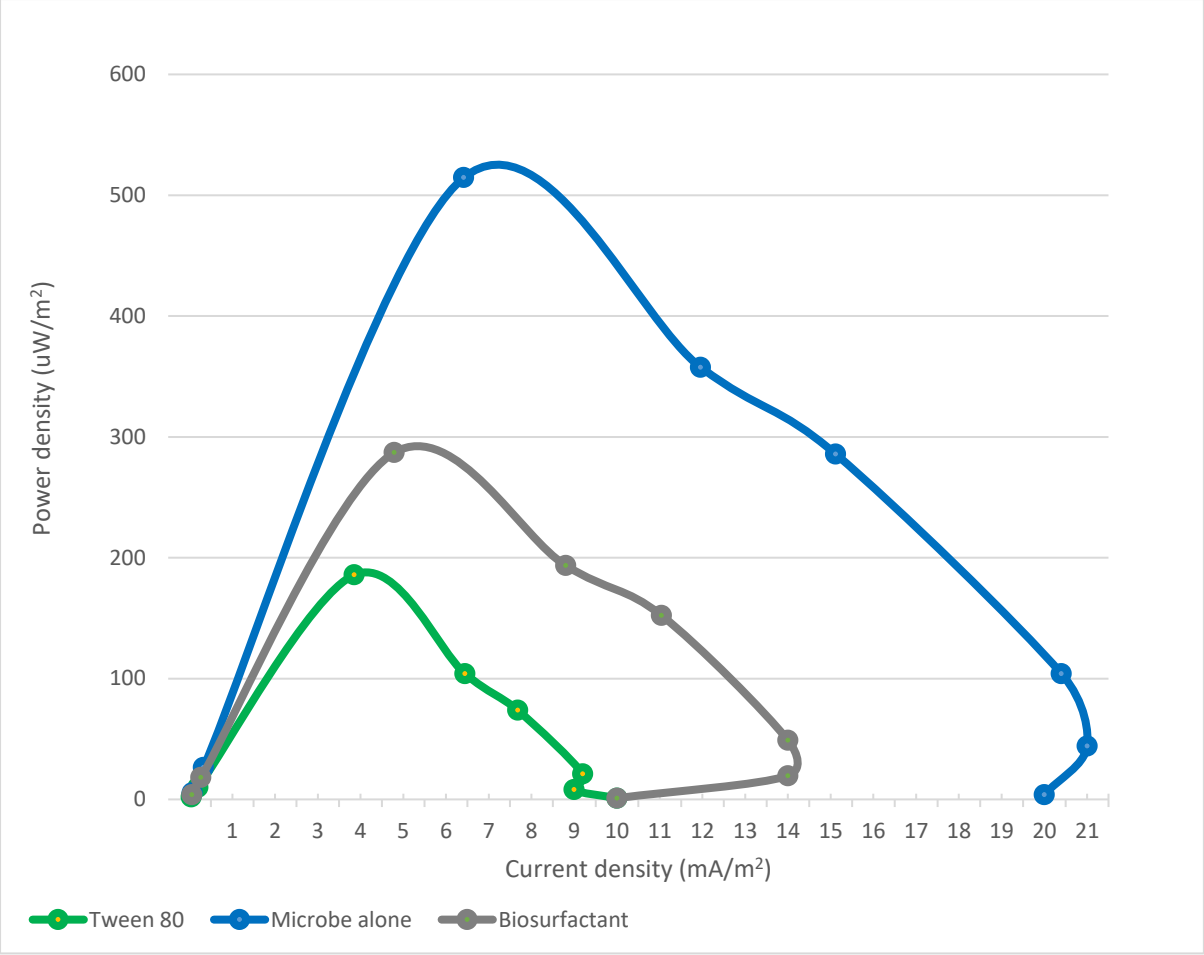


Figure 5.3 Power density (uW/m²) outputs from H-type MFCs inoculated with petroleum hydrocarbon polluted groundwater microcosms with benzene and phenanthrene as co-substrate in three different experimental setups: Microcosms alone, the microcosms supplemented with biosurfactant and the microcosms supplemented with chemical surfactant (Tween 80).

5.5 Degradation rate and degradation efficiency of phenanthrene in groundwater MFC

The degradation rate of phenanthrene per day in the different MFCs in Figure 5.4 and Figure 5.5, shows that the MFCs that was supplemented with biosurfactant had the highest degradation rate of 0.28 mg per day. This was closely followed by the MFCs supplemented with Tween 80 that recorded a degradation of 0.21 mg per day. While the MFC with only the microcosm without surfactant supplement had 0.13 mg per day while the negative control that had neither microcosm nor surfactant had the least degradation rate of 0.015 mg per

day. The biosurfactant from the sediment isolates A in Figure 3.16 used as the biosurfactant supplement for the MFC performed better than the chemical surfactant (Tween 80). This shows that the biosurfactant has the potential of being a better replacement to chemical surfactants as it did not just perform better than the chemical surfactant but is also a sustainable alternative and could be more environmentally friendly. In comparison to the outcome of Adelaja (2015) experiment that lasted 155 days for a degradation of over 90% of 30 mg that was equivalent of 0.194 mg degradation rate per day, the formulated groundwater supplemented with biosurfactant performed better. The combination of the groundwater microcosm with the biosurfactant has the potential of enhancing the degradation of phenanthrene in MFC remediation approach. The overall result shows that the experimental set up with the microcosm and supplemented with surfactant (biosurfactant and Tween 80) had the first and second highest degradation rate and highest degradation efficiency for phenanthrene, The bioreactor inoculated with only the microcosm with no surfactants supplementation had the third highest degradation rate and degradation efficiency for phenanthrene while the negative control that was neither inoculated with the microcosm nor supplemented with surfactant had the least degradation rate and the least degradation efficiency. While the microcosm alone degraded phenanthrene, the surfactant (either biosurfactant or tween 80) supplementation were observed to have enhanced the degradation of phenanthrene by the microcosm. On the other hand, the negative control that neither had the microcosm inoculated nor surfactant supplementation had the least degradation rate and degradation efficiency for phenanthrene. The enhanced degradation rates of phenanthrene by the microcosms in the presence of surfactant could be attributed to increase bioavailability of the insoluble phenanthrene to the microcosms for degradation. Surfactants are said to play a key role in the degradation of insoluble substrates by enhancing

the interaction between insoluble substrates and microorganisms that helps in facilitating substrate transport to the cell (Kavitha et al., 2014; Varjani and Upasani, 2016). Although not supplemented with any surfactant, it was observed that the microbes alone also had a significant degradation rate. As seen in **Figure 3.19** one of the groundwater strains that made up the microcosm for this experiment produced some surfactant. This could have enhanced the rate of phenanthrene degradation. On the other hand, the negative control MFC that contained only the pollutants with neither microcosm nor surfactant had the least degradation for phenanthrene. The overall result showed that the integration of MFC petroleum hydrocarbon degrading microcosms and supplemented with biosurfactant could enhance the degradation of phenanthrene.

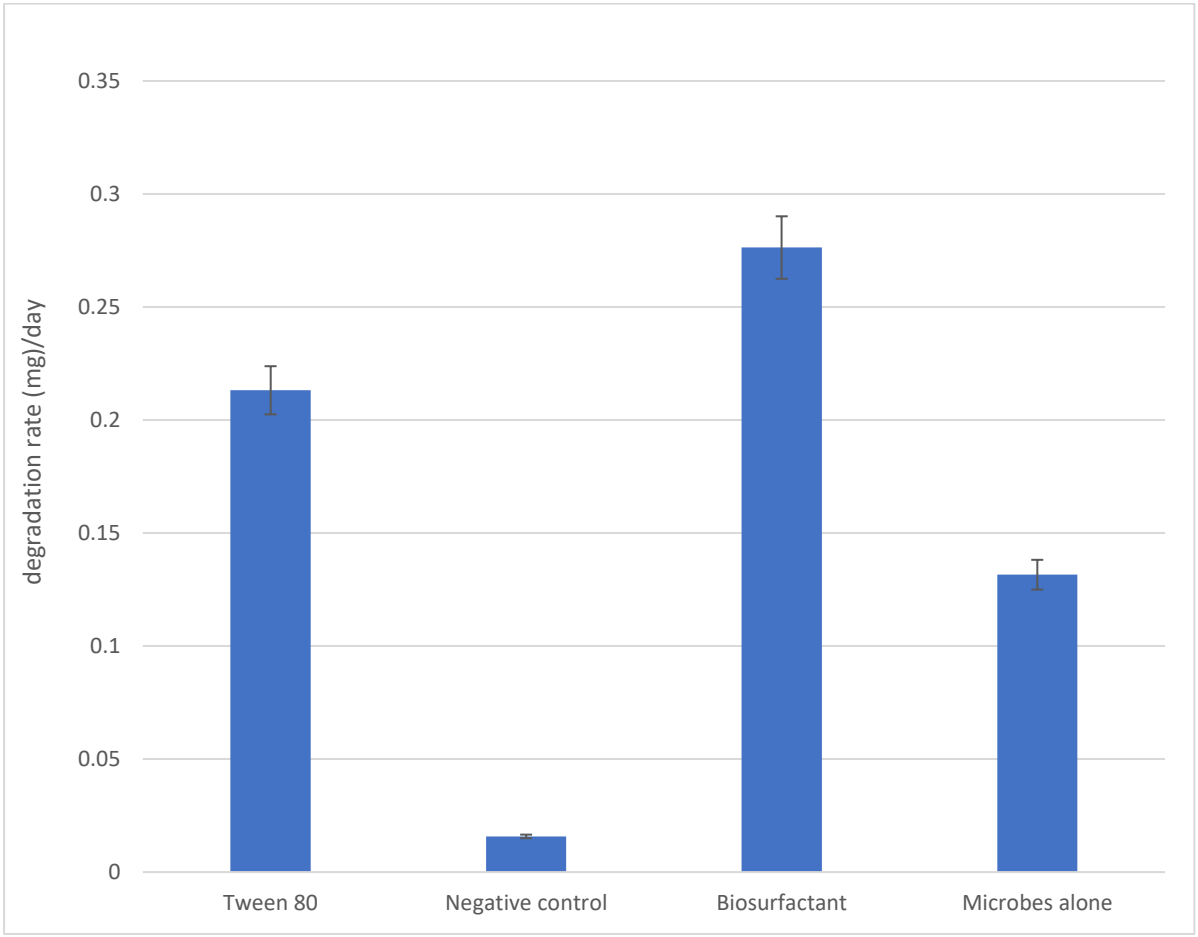


Figure 5.4 Phenanthrene degradation rate (mg)/day) in a H-type MFCs inoculated with microcosm isolated from petroleum hydrocarbon polluted groundwater, with benzene and phenanthrene as co-substrate. The experimental setups: microcosms supplemented with chemical surfactant (Tween 80), Negative control containing only the pollutants with neither microcosm nor surfactant supplement, microcosms supplemented with biosurfactant and Microcosms alone. .

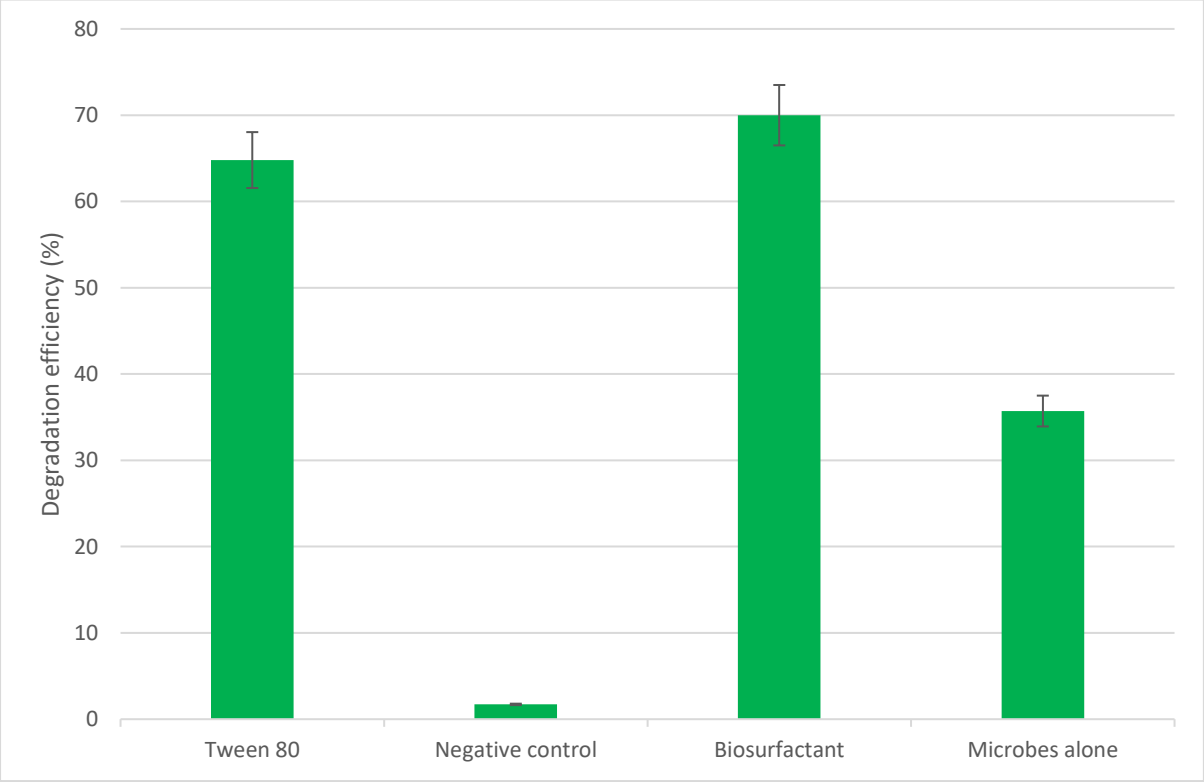


Figure 5.5 Phenanthrene degradation efficiency (%) in a H-type MFCs inoculated with microcosm isolated from petroleum hydrocarbon polluted groundwater, with benzene and phenanthrene as co-substrate. The experimental setups include microcosms supplemented with chemical surfactant (Tween 80), Negative control containing only the pollutants with neither microcosm nor surfactant supplement, microcosms supplemented with biosurfactant and Microcosms alone.

5.6 Degradation rate and degradation efficiency of benzene in groundwater MFC.

The degradation rate of benzene per day in the different MFCs set up in Figure 5.6 differs from one setup to the other. The MFC that was supplemented with biosurfactant had the

highest degradation rate of 0.85 mg per day. This was followed by the negative control with 0.62 mg/day while the MFCs supplemented with Tween 80 had a degradation rate of 0.55 mg/day the microbes alone had 0.37 mg/day being the least degradation rate... When the degradation rates in Figure 5.4 and Figure 5.6 for phenanthrene and benzene is compared it is interesting to observe that the negative control with almost a zero percent phenanthrene degradation, had the second highest degradation rate for benzene The reason the negative control may have had a significant degradation for benzene could be because the microorganisms in the water were able to utilise benzene as the substrate than the phenanthrene since microorganisms have been reported to easily degrade shorter and simpler structure petroleum hydrocarbons (Van Hamme et al., 2003). Furthermore, the obvious low desorption and dissociation of phenanthrene in solution may have also led to only the adsorption of benzene onto the electrode thereby resulting in lower concentration in solution. On the other hand, the low degradation rates of benzene in the Tween 80 supplemented MFC might be because the Tween 80 enhanced the growth of species within the microcosm that could degrade phenanthrene into benzene as one of its metabolites since complex petroleum hydrocarbon can be degraded in different pathways (Wartell et al., 2021). This could be that the more phenanthrene was degraded the more benzene was produced. On the other hand, biosurfactant supplemented MFC had the highest degradation rate and degradation efficiency for both benzene and phenanthrene which could be that the biosurfactant enhanced the degradation of both phenanthrene and benzene. The overall result in Figure 5.6 and Figure 5.7 showed that the MFC inoculated with the microcosm formulated from petroleum hydrocarbon polluted groundwater and supplemented with biosurfactant had the highest degradation rates and degradation efficiencies for both phenanthrene and benzene.

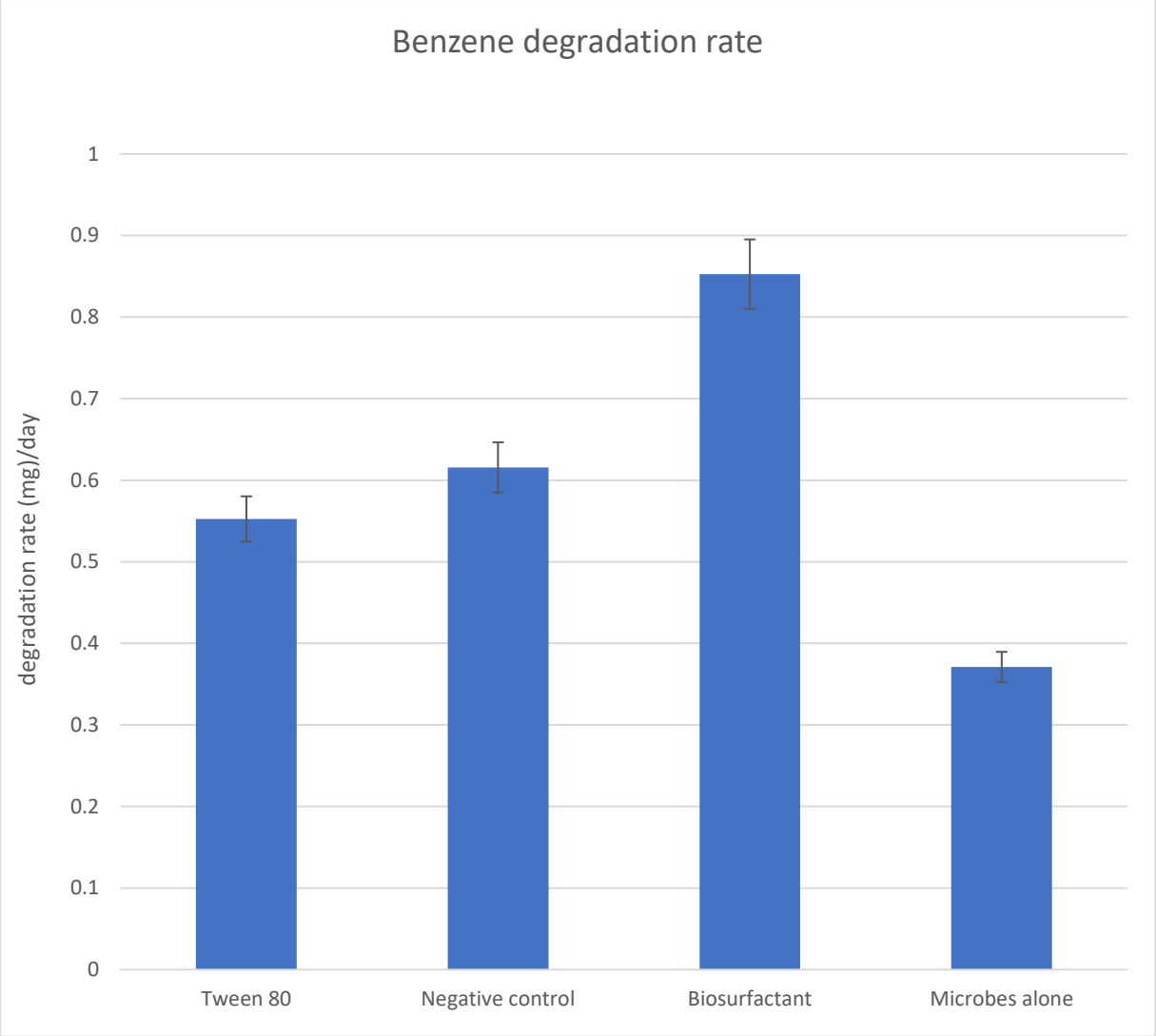


Figure 5.6 Benzene degradation rate mg/day in a H-type MFCs inoculated with microcosm isolated from petroleum hydrocarbon polluted groundwater, with benzene and phenanthrene as co-substrate. The experimental setups include microcosms supplemented with chemical surfactant (Tween 80), Negative control containing only the pollutants with neither microcosm nor surfactant supplement, microcosms supplemented with biosurfactant and Microcosms alone

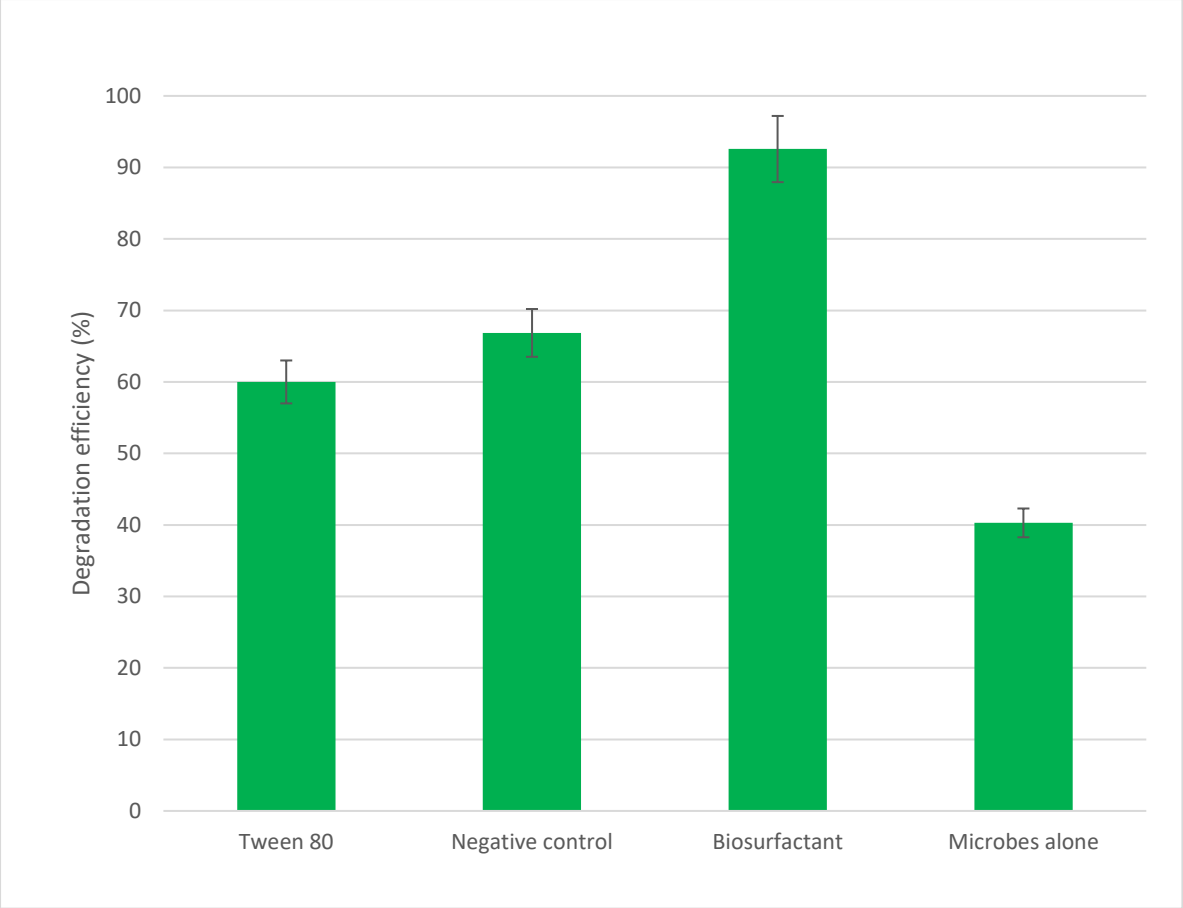


Figure 5.7 Benzene degradation efficiency (%) in a H-type MFCs inoculated with microcosm isolated from petroleum hydrocarbon polluted groundwater, with benzene and phenanthrene as co-substrate. The experimental setups include microcosms supplemented with chemical surfactant (Tween 80), Negative control containing only the pollutants with neither microcosm nor surfactant supplement, microcosms supplemented with biosurfactant and Microcosms alone.

5.7 Conclusion

From the result above, it can be concluded that supplementing the microcosm with the biosurfactant extracted from the sediment isolate A in Figure 3.16 in an MFC remediation system of petroleum hydrocarbons (benzene and phenanthrene) can be more effective than a systems consisting of a chemical surfactant (Tween 80) or the microcosm alone or negative control. This newly discovered biosurfactant can potentially contribute positively to petroleum hydrocarbon bioremediation and possibly oil recovery.

Chapter 6: Comparison the effects of different microbial fuel cell configuration (air breathing and snorkel) on petroleum hydrocarbon degradation

6.1 Overview

Sediment like other environmental matrices have had several pollutants enter and bioaccumulate that are threatening the health of the water bodies due to their toxicity (Cheng et al., 2021a; Zhang et al., 2020; Cheng et al., 2021b). The high concentration of these pollutants and their low degradation made it necessary for more effective ways of remediating these pollutants. Sediment microbial fuel cell has been employed in recent times as a cost-effective and ecofriendly alternative for the remediation of pollutants from sediment (Serra et al., 2021) with several successes reported (Morris et al., 2012; Ewing et al., 2017; Yan et al., 2017). There have been different MFC designs made in an effort to optimise the system. A snorkel is one of the simplest MFC designed with a direct coupling of both the anode and the cathode (Erable et al., 2011). This can simply be described as a short circuit MFC and when short-circuited there is zero voltage between the anode and the cathode thereby leading to no power but works at the possible maximum current an MFC can produce. Because of its ability of sustaining maximum current between the cathode and the anode, the system can achieve a maximum reaction rate according to its capacity, thereby making the snorkel suitable for raising electrochemical reaction rate that is aimed at pollutant removal rather than production of electricity (Santoro et al., 2017). The use of snorkel has been reported to be effective in the remediation of petroleum hydrocarbon (Viggi et al., 2015; Matturro et al., 2017). On the other hand, an air breathing MFC is a design that does not have cathode and anode coupling but has a cathode with a diffusion layer that controls oxygen diffusion from the air to the cathode thereby removing external aeration demand (Liu et al., 2015). In air breathing MFC, the cathode has direct contact with the electrolyte on one side and on the other side, also has direct contact with air (Vogl et al., 2016). In attempts to optimise the system, there have several approaches to decipher what improvements need to

be made and in choosing the most suitable material and designs that can give the best result for the overall system. In this quest, this chapter compared results for two different electrode designs (snorkel and air breathing electrode) to ascertain the most suitable for the degradation of petroleum hydrocarbon pollutants (benzene and phenanthrene) in a laboratory experiment that mimics a sediment matrix.

6.2 Voltage output sediment MFC

From **Figure 6.1** the sediment voltage output for the air breathing MFC for the sediment over time shows an initial declining voltage that after recording a negative voltage suddenly ascends to a peak voltage of 141.84 (mV) and then declines. It is important to note that even with an upward and downward trend there is a fluctuation of voltage within the trends this is most likely to be due to the fluctuation of oxygen flowing from the environment into the bioreactor that affects the degradation of activities in the system as a result affects the voltage output. The trend of this voltage output is completely different when compared with those in Chapters 4 and 5 where a H-type MFC was used. This may be because air breathing electrode depends on surrounding oxygen to function (Vogl et al., 2016) and the fluctuation of oxygen may be the major, if not the only reason, of the observed fluctuation in voltage output. After the upward and downward trends, the voltage declines towards a zero voltage in about the 140th hour without ascending as previously observed in Figure 6.1. This may be due to biofouling as it was same time that biofilm formation was visibly observed on the surface of the electrode. Biofouling is said to be one of the challenges of air breathing electrode and its impact becomes prominent when the system operates for a relatively long period of time. Biofouling is said to, not just decrease the cathodic catalytic activity but also, decrease the overall stability of the MFC over time (Li et al., 2023).

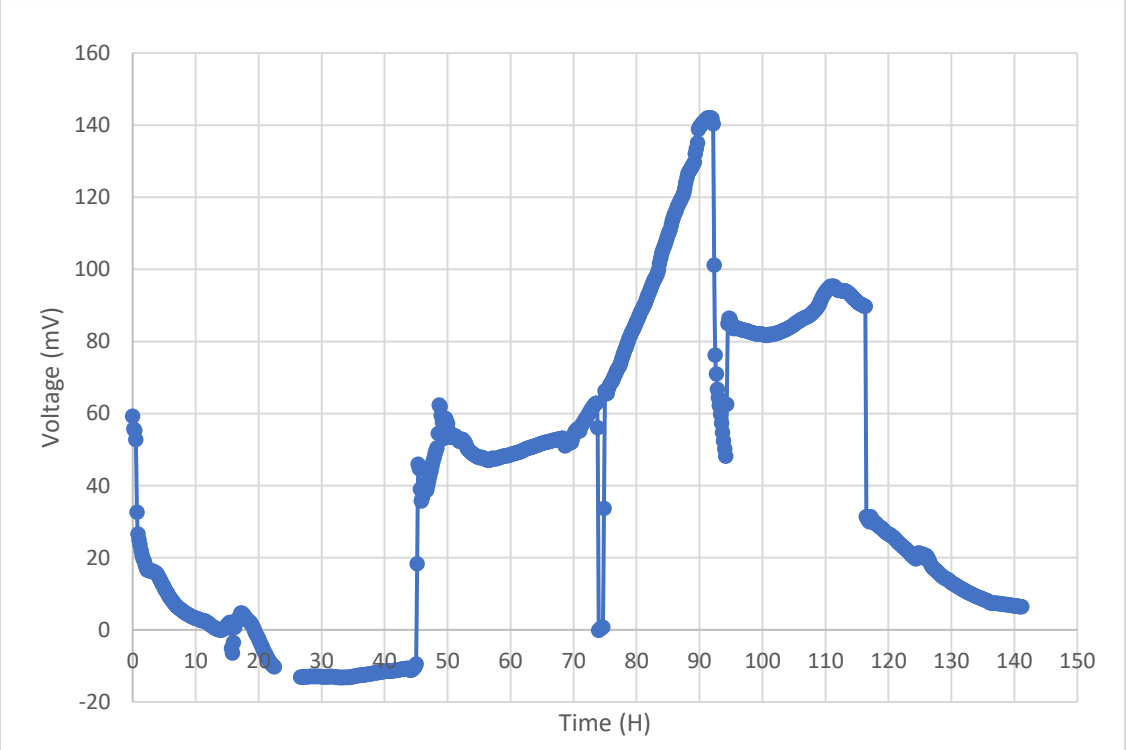


Figure 6.1 Voltage (mV) output over time from sediment microbial fuel cell using air breathing electrode inoculated with isolates from petroleum hydrocarbon polluted sediment microcosm with benzene and phenanthrene as co-substrate.

6.3 Polarisation curve of sediment MFC

The slope of the polarisation curve in **Figure 6.2** shows the internal resistance of the air breathing electrode. When compared with the polarisation curve in Chapter 5 with a different type of MFC and matrix it can be seen that the trend is also different. While there is difference in matrix and MFC design, the curve in Figure 6.2 indicates that the internal resistance can be said to be majorly due to ohmic losses. This may also be as the result of oxygen fluctuation since air breathing electrode depends on surrounding oxygen to function (Vogl et al., 2016).

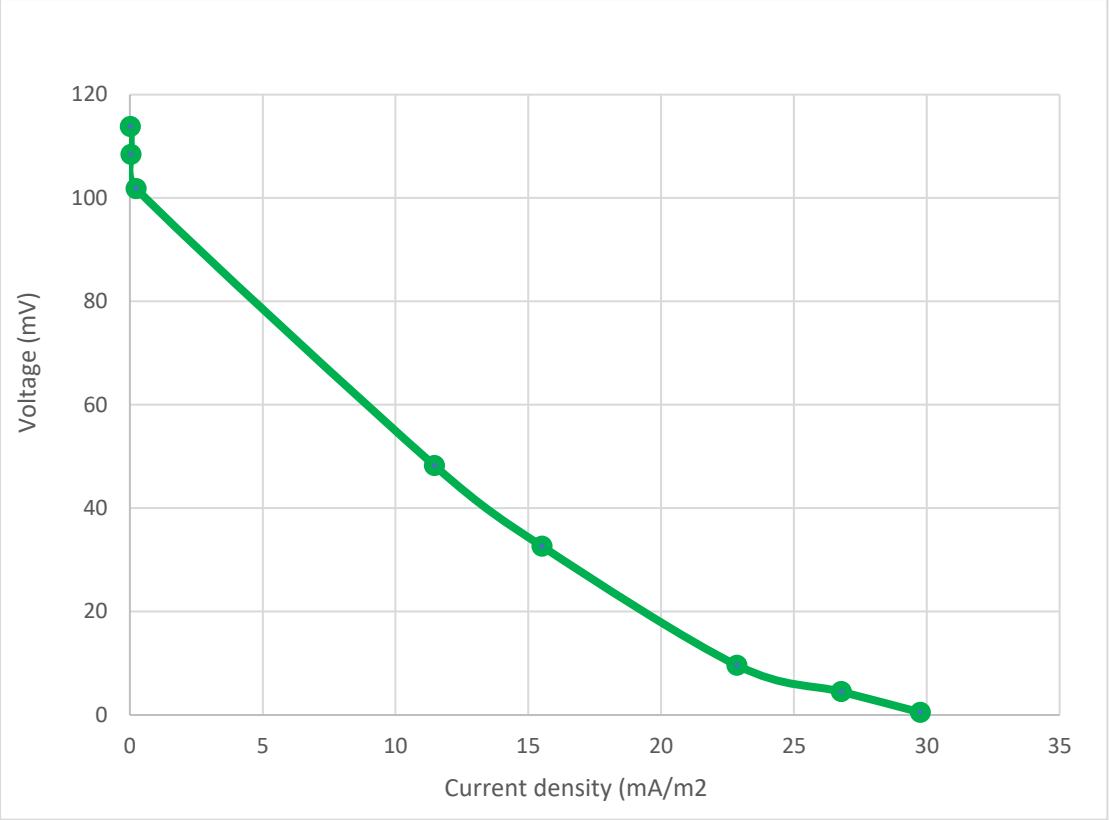


Figure 6.2 Polarisation curve from sediment microbial fuel cell using air breathing electrode inoculated with microcosm isolates from petroleum hydrocarbon polluted sediment with benzene and phenanthrene as co-substrate.

6.4 Power density of sediment MFC

Although the major target of this research is the degradation of the pollutants, but when compared with the power density output of the H-type MFC in Chapters 4 and 5, the power density of the air breathing in Figure 6.3 is higher. This is similar to other reports and indeed air breathing electrode has been reported to have higher power density because of its lower internal resistance (Liu et al., 2006; Liu et al., 2004)

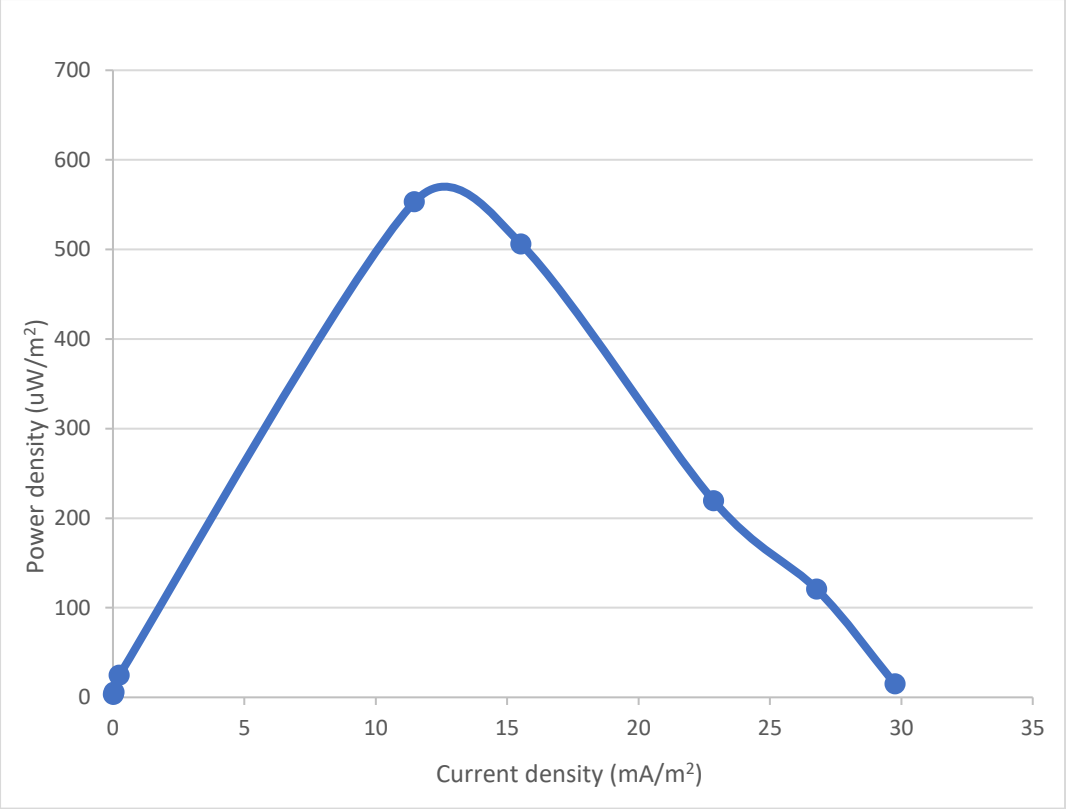


Figure 6.3 Power density (uW/m²) from sediment microbial fuel cell using air breathing electrode inoculated with isolates from petroleum hydrocarbon polluted sediment microcosm with benzene and phenanthrene as co-substrate.

6.5 Degradation rate and degradation efficiency of phenanthrene of sediment MFC

The result in Figure 6.4 shows the degradation rate per day for phenanthrene in the different MFC set ups and negative control both at the liquid phase (water) that is at the surface of the bioreactor and the solid phase (soil) at the bottom of the bioreactor. In the solid phase, the air breathing MFC had the highest degradation rate with 0.613 mg per day this was followed the snorkel MFC with 0.044 mg per day while the natural attenuation had the least degradation rate of 0.00175 mg per day. On the other hand, natural attenuation had the highest degradation of 0.175 mg per day in liquid phase followed by snorkel with 0.01045 mg per day while the air breathing had the least with 0.005175 mg degradation rate per day. From the results in both MFCs, it shows that MFC can enhance the degradation of petroleum

hydrocarbons under anaerobic condition compared to natural attenuation. The noticeable degradation at the liquid phase for natural attenuation can be due to aerobic degradation since the liquid phase is at the surface where oxygen is more abundant. As there was higher microbial growth and activities in the MFCs inoculated with microcosms, the turbidity created by these (growth) and metabolites from these activities may have resulted to lower diffusion of oxygen in the liquid phase thereby leading to the lower degradation of phenanthrene in both MFCs in the liquid phase compared to that of the natural attenuation. Furthermore, as there is close to zero degradation, desorption and dissociation of phenanthrene in solid phase for natural attenuation, this may have lowered the diffusion of solubilise phenanthrene molecules to the liquid phase from the solid phase thereby, increasing the degradation at the liquid phase. Figure 6.5 air breathing MFC had the highest degradation efficiency of about 70% followed by snorkel with 6.01% and 0.0116% for natural attenuation at the solid phase. The liquid phase had lower degradation efficiencies for all the set-ups; snorkel had the highest of 39.64% followed by natural attenuation with 29.3% and air breathing with degradation efficiency of 24.39%. The relationship between the degradation rate and degradation efficiency in the liquid phase is neither directly proportional nor inversely proportional. This may be due to factors that include but not limited to oxygen diffusion and the type of cathodic electrode that was used in the MFC set-ups.

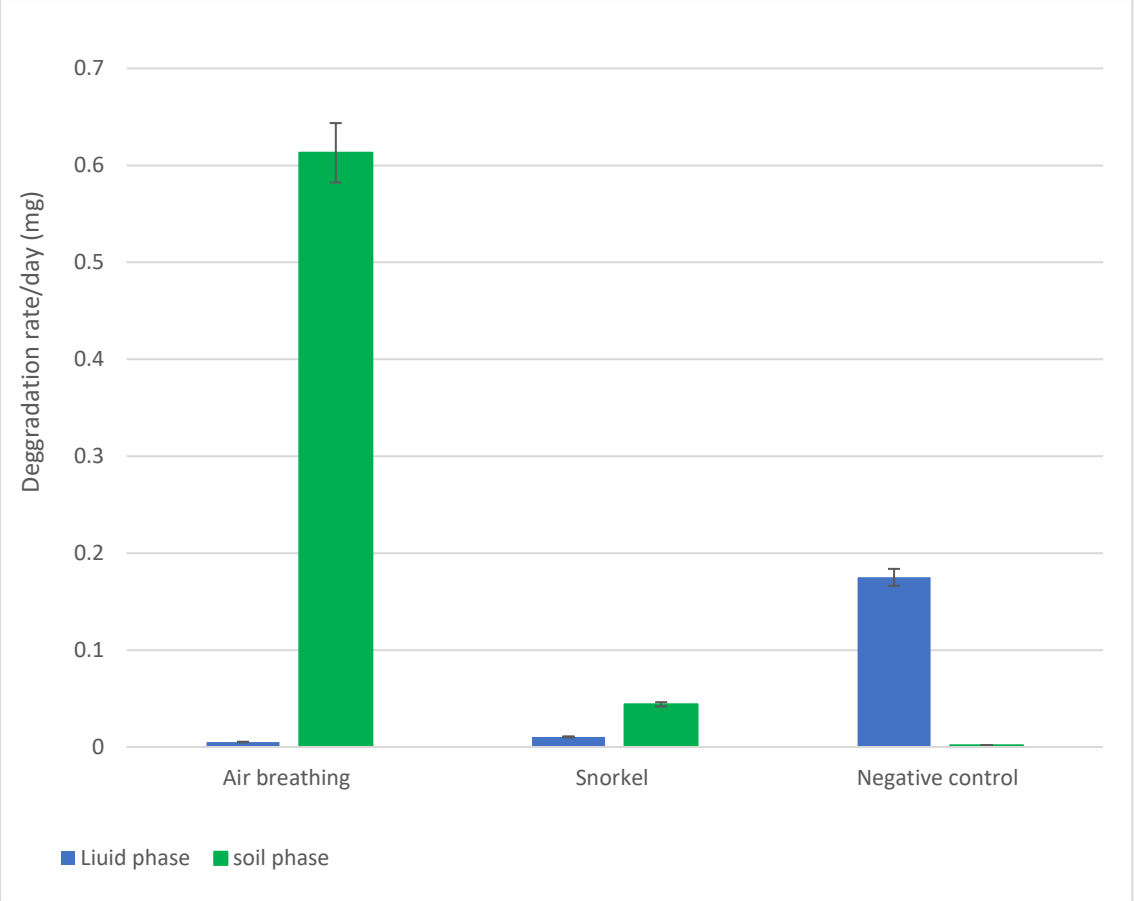


Figure 6.4 Phenanthrene degradation rate/day (mg) in petroleum hydrocarbon polluted sediment microcosm with benzene and phenanthrene as co-substrate for the sediment MFCs with air breathing and snorkel electrode. and the negative control had only the pollutants with neither electrode nor microcosm.

6.6 Degradation rate and degradation efficiency of benzene of sediment MFC

The degradation rate of benzene in Figure 6.6 shows that natural attenuation had the highest degradation rate of 0.565 mg per day followed by snorkel MFC with 0.44 mg per day while air breathing had the least with 0.04 mg per day in the solid phase while there was no detectable concentration in the liquid phase for all the set-ups. The high degradation rate of benzene in the natural degradation may be as a result of microbial preference of a less complex and bioavailable compound than phenanthrene. However, the low degradation rate in the MFCs may not be the true degradation rate of benzene in these bioreactors owing to the fact that phenanthrene being a complex compound may have been degraded into benzene as one of its metabolites. This may be the reason that the air breathing MFC with the highest phenanthrene degradation had the least degradation or benzene. This could mean that the more

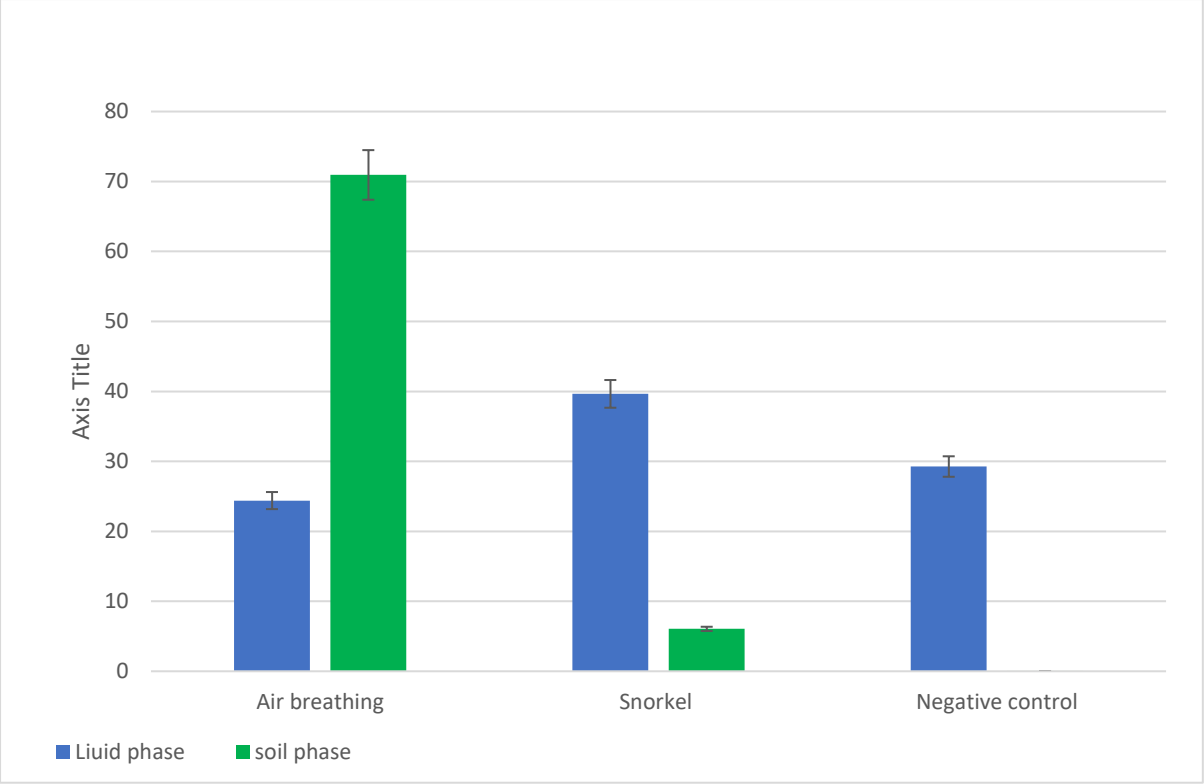


Figure 6.5 Phenanthrene degradation efficiency (%) in sediment microbial fuel cell using air breathing and snorkel MFC configuration inoculated with microcosm (11 isolates) from petroleum hydrocarbon polluted sediment microcosm with benzene and phenanthrene as co-substrate for the sediment MFCs with air breathing and snorkel electrode. The negative control had only the pollutants with neither electrode nor microcosm.

phenanthrene was degraded, the more benzene could have been produced as one of the metabolites. Moreover, benzene was the sole source of carbon that was used as the screening pressure in selecting these microorganisms; this gives an insight in the possibility of these organisms possessing the mechanism that degraded phenanthrene to benzene. This can be likened to a report by Guo et al. (2020) that the presence of an electrode can promote electrochemical oxidation able to enhance the conversion of high-molecular-weight polycyclic aromatic hydrocarbons to small-molecular weight aromatics or linear-aliphatic hydrocarbon in a system. The degradation efficiency in Figure 6.7 shows that snorkel had the highest degradation efficiency of 39.4% followed by natural attenuation with 30.7% while air breathing had the least with 3.7%. The relationship trend between degradation rate and degradation

efficiency is similar to that observed in the degradation of phenanthrene in the liquid phase above.

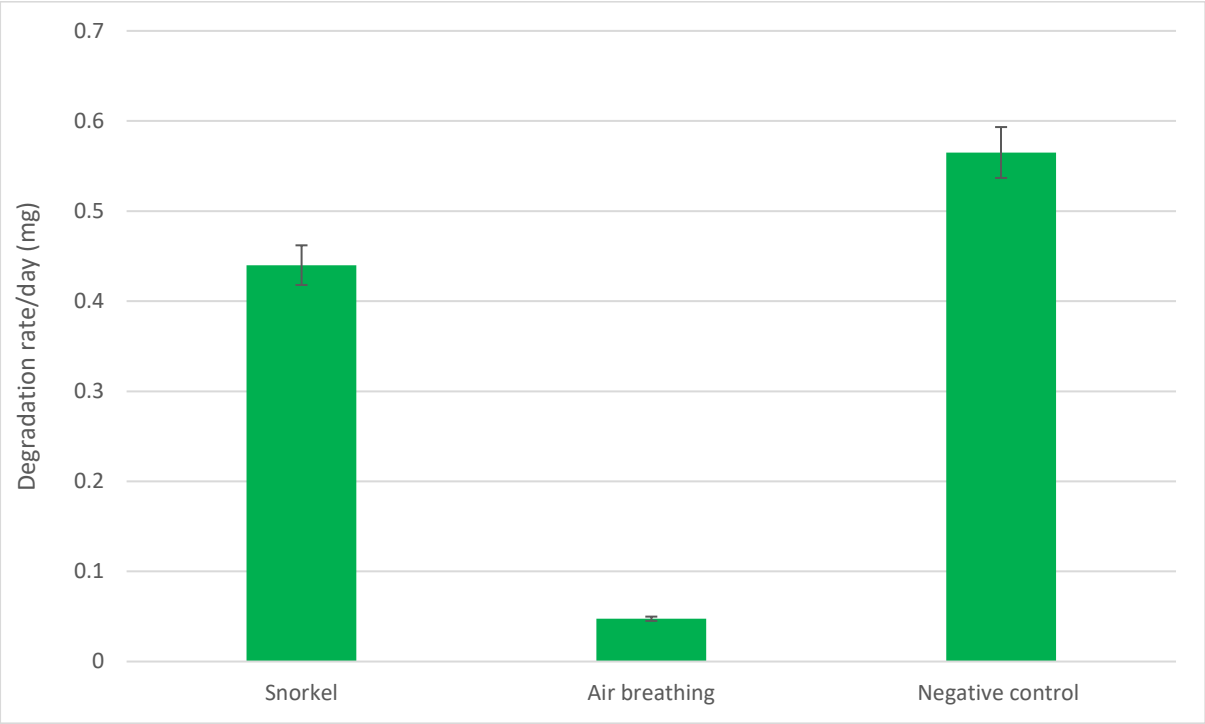


Figure 6.6 Benzene degradation rate/day (mg) in sediment microbial fuel cell using air breathing electrode inoculated with isolates from petroleum hydrocarbon polluted sediment microcosm with benzene and phenanthrene as co-substrate for the sediment MFCs with air breathing and snorkel electrode and negative control that had only the pollutants with neither electrode nor microcosm.

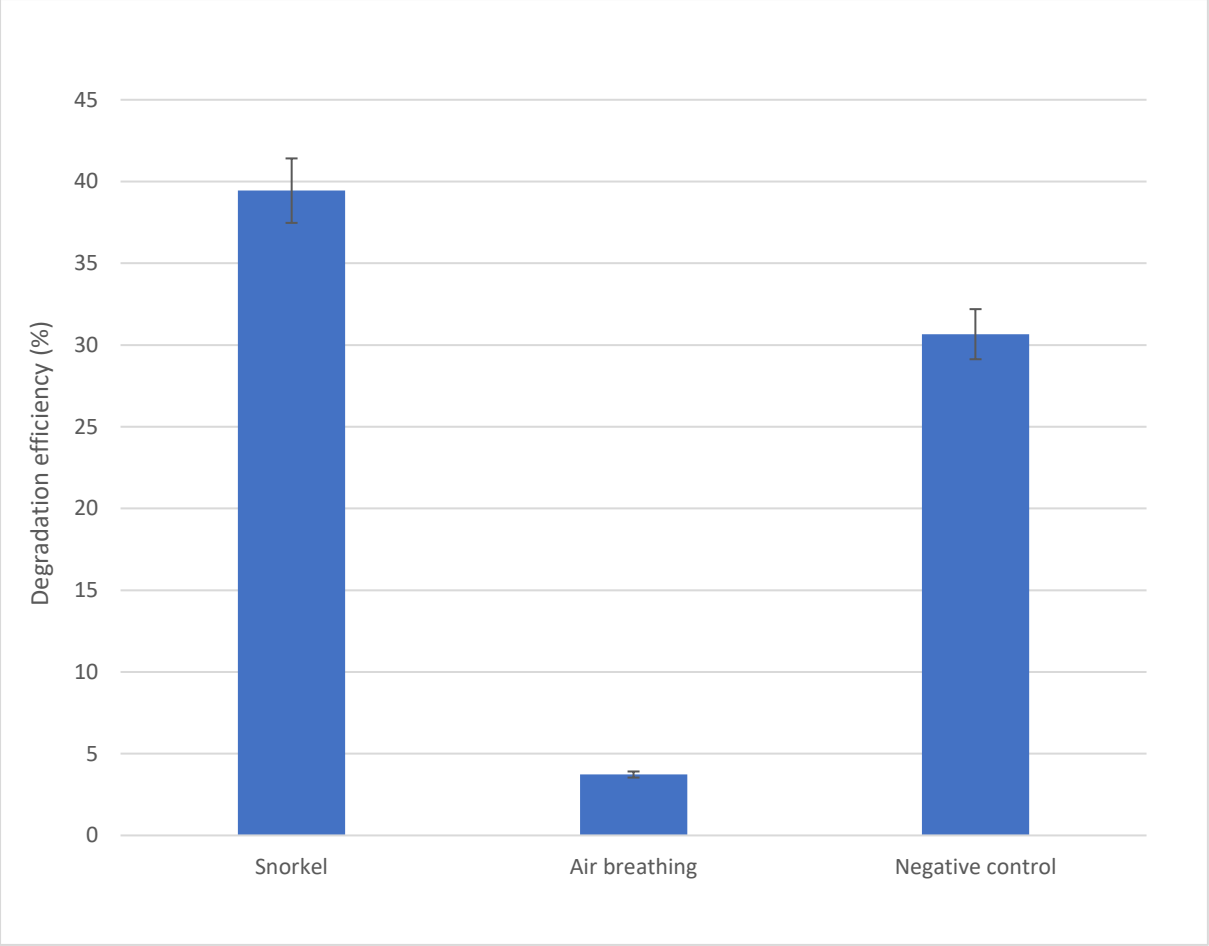


Figure 6.7 Benzene degradation efficiency (%) in sediment microbial fuel cell using air breathing electrode inoculated with isolates from petroleum hydrocarbon polluted sediment microcosm with benzene and phenanthrene as co-substrate for the sediment MFCs with air breathing and snorkel electrode. The negative control had only the pollutants with neither electrode nor microcosm.

6.7 Conclusion

From the result above it can be concluded that the MFC system (snorkel and air breathing) with microcosm have the potential to effectively accelerate the degradation of petroleum hydrocarbon from polluted sediment than natural attenuation when deploy in the field. The integration of the microcosm with MFC can speed up the degradation of phenanthrene making this an effective approach in the remediation of petroleum hydrocarbons.

Chapter 7: Effects of biochar on electrode Radius Of Influence (ROI) in soil microbial fuel cell

7.1 Overview

7.2. Biochar

Biochar is a carbon rich material that is produced by the converting carbonaceous compound or biomass at high temperatures between 600^0C and 800^0C in the absence of oxygen (Gupta et al., 2020). Biochar as an energy effective carbon material has microporosity, high ion exchange potential and a large surface area. Its microporosity characteristics has given this material the potential of its suitability in environmental application due to abundant feedstock and low cost (Ahmad et al., 2014; He et al., 2022). Biochar has been applied in remediating soil from organic contaminants and heavy metals. This is due to its ability to effectively adsorbs these contaminants from the soil. Biochar adsorption of pollutants is said to occur through interactions that include acid–base interaction, hydrogen binding, surface complexation, π-π interaction and electrostatic attraction (Zhang et al., 2013). Biochar has been reported to enhance the degradation of petroleum hydrocarbon by increasing the relative abundance of PAHs degraders thereby resulting in increase in PAHs degradation in soils (Kong et al., 2018). Zhang et al. (2020) reported an increase in PAHs biodegradation rate in soils with biochar compared to the soils without biochar. These and several studies have reported the positive effects of biochar in the biodegradation of petroleum hydrocarbons. Recently, biochar has also found application in microbial fuel cell as electrode or as catalyst of the system for contaminants remediation and power generation (Saran et al., 2023). In MFC, biochar plays a key role in transporting electrons through electron shuttles and electrical conductivity, thereby enhancing the MFC system by increasing the scope and efficiency of microorganisms' metabolism (Shi et al., 2019). The result in this chapter gives an insight on the effect of biochar on the radius of influence of electrons in a soil microbial fuel cell by comparing soil with biochar and soil without biochar, with both systems inoculated with

microcosm isolated from petroleum hydrocarbon polluted soil. A negative control that contained neither the soil microcosm nor biochar was set up

7.2 Voltage output of soil MFC

The voltage output in Figure 7.1 that the MFC without biochar had the highest voltage peak current of 526 (mV) while the MFC with biochar had 375 mV. While the MFC without biochar has a higher voltage output, both systems have similar trend from higher to declining and stabilizing within voltage ranges.

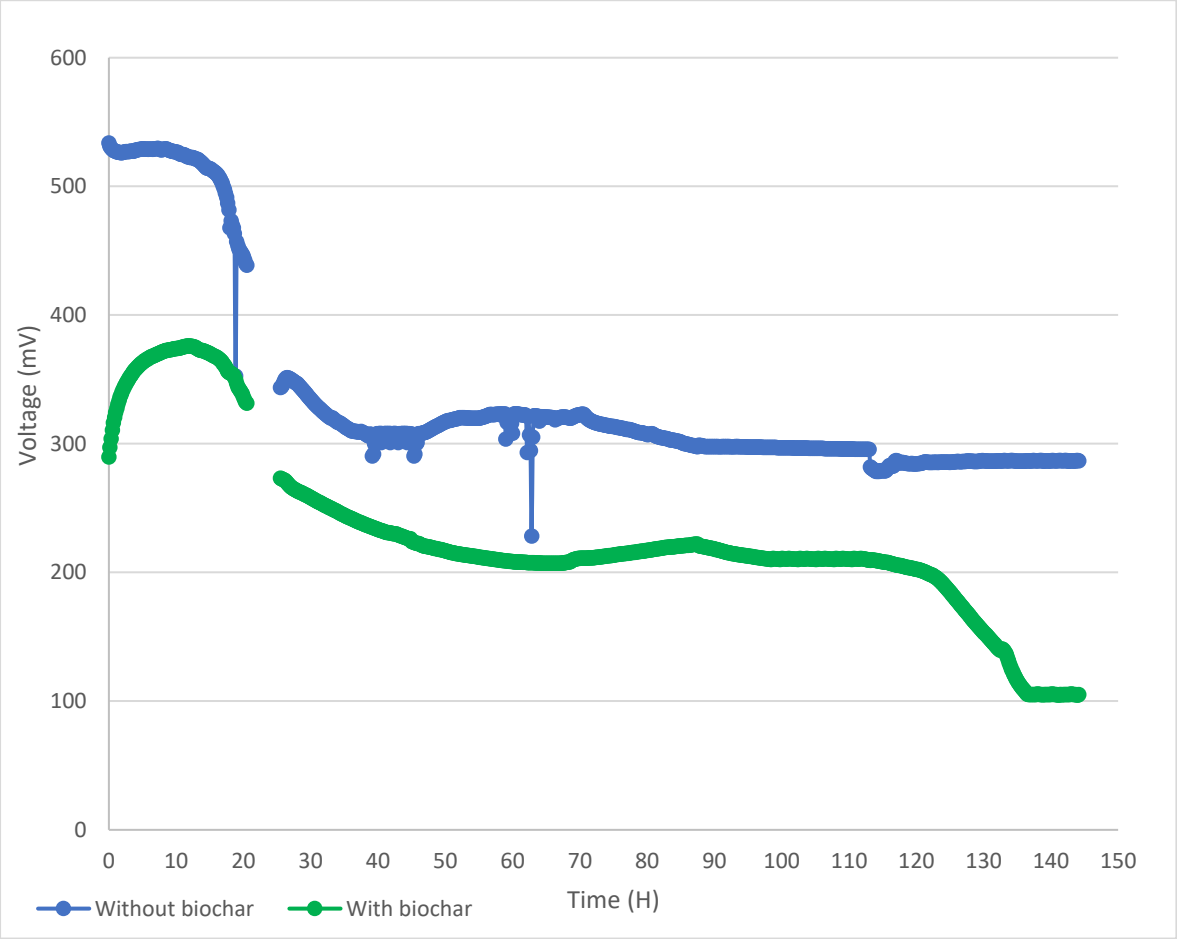


Figure 7. 1 Voltage (mV) output over time from soil microbial fuel cell inoculated with isolates from petroleum hydrocarbon polluted soil microcosms with benzene and phenanthrene as co-substrate in two different experimental setups: soil microbial fuel cell supplemented with 5% biochar and soil microbial fuel cell without biochar.

7.3 Polarisation curve of soil MFC

The polarisation curve in Figure 7.2 shows different trends in the polarisation curves in the two MFC depicting differences in internal resistance of the system. Probably the use of biochar influenced the reduction of the ohmic losses in the MFC that contained biochar. Since the polarisation curves help in giving insight to the overall system, this will help in the optimization of the overall system.

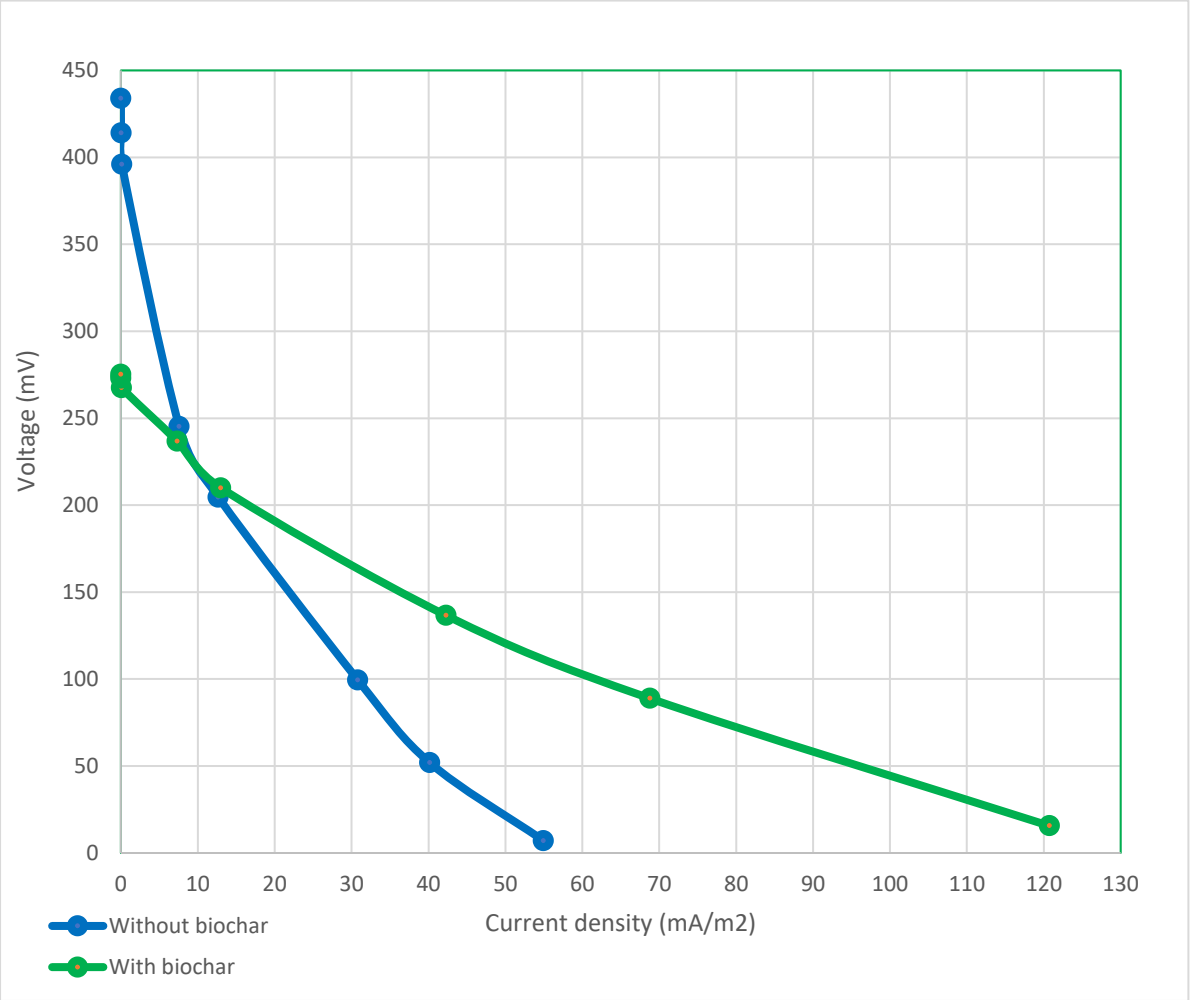


Figure 7.2 Polarisation curve from soil microbial fuel cell inoculated with isolates from petroleum hydrocarbon polluted soil microcosms with benzene and phenanthrene as co-substrate in two different soil microbial fuel cells experiment with one supplemented with 5% biochar and the other soil microbial fuel cell without biochar.

7.4 Power density of soil MFC

The power density in Figure 7.3 shows that the MFC with biochar had a peak power density o 6114.28 (uW/m^2) while the MFC without biochar had 3065.1 (uW/m^2). The power density of the MFC with biochar can be seen to be almost double that of the MFC without biochar. This is not surprising as biochar has been reported to play a key role in transporting electrons through electron shuttles and electrical conductivity, thereby enhancing the MFC system by increasing the scope and the efficiency of microorganisms' metabolism (Shi et al., 2019).

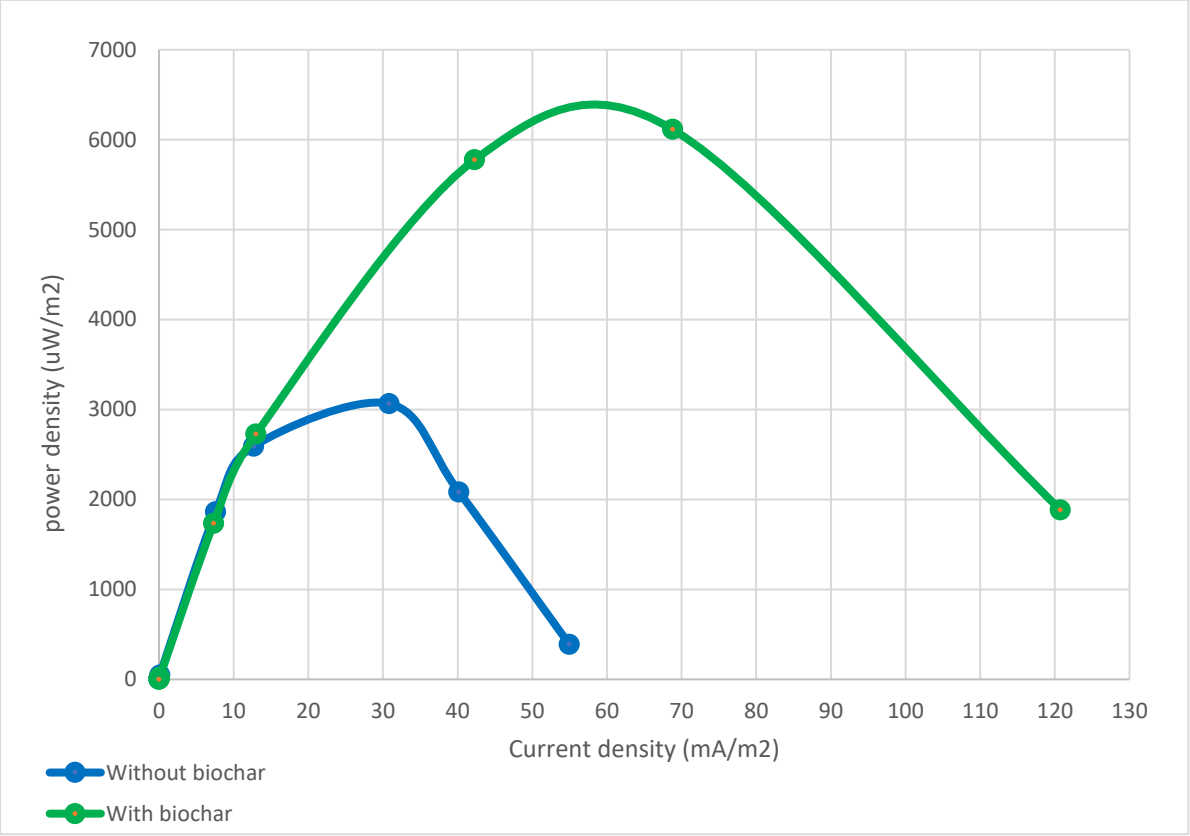


Figure 7. 3 Power density (uW/m2) from soil microbial fuel cell inoculated with isolates from petroleum hydrocarbon polluted soil microcosms with benzene and phenanthrene as co-substrate in two different set experimental set ups: soil microbial fuel cell supplemented with 5% biochar and soil microbial fuel cell without biochar.

7.5 Degradation rate and degradation efficiency of soil MFC

While it took the two MFCs containing the microcosm (with biochar and without biochar) about 6 days to degrade both the 100 mg of phenanthrene and 200 mg of benzene below the 0.05 mg/L for benzene and 0.005 mg/L phenanthrene detectable limit on the HPLC, there was no significant degradation in the negative control. The degradation rate for both phenanthrene by the microcosm either supplemented with biochar or not is a significant improvement to a similar experiment carried out by Adelaja (2017). This may be as the result of the combination of the microcosms that showed improved performance in electrochemical activity when compared with *S. oneidensis* in Chapter 3. On the other hand, the negative control had about 1.05 mg and 0.0058 mg degradation rate/day for benzene and phenanthrene respectively. The degradation efficiency for the MFCs was 0.178% and 0.131% for benzene and phenanthrene respectively.

7.6 Conclusion

From the result above it can be concluded that the MFC assays with microcosm and either with biochar or without biochar can accelerate the degradation of petroleum hydrocarbon from polluted soils than natural attenuation. The integration of the microcosm made up of species with good to excellent electrochemical activity that are petroleum degraders shows great potential in petroleum hydrocarbon remediation.

Chapter 8: Summary, Conclusion and Perspectives

8.1 Summary

The overall aim of the research presented in this thesis was to develop and test bioelectrochemical systems-based petroleum hydrocarbon remediation technology at laboratory scale in environmentally-relevant conditions with a view for field deployment. To achieve this aim, five major studies were carried out from chapter 3-7.

Chapter 3 was focused on bioprospecting for isolates that are petroleum hydrocarbon degraders, facultative anaerobes with good electrochemical activity and desired but not a mandatory criterion the production of biosurfactant. The samples used for bioprospecting were collected from prolonged petroleum hydrocarbon polluted matrices (soil, sediment and groundwater) in the Niger-Delta region of Nigeria. Using two culture- based approaches and screening pressures 200 mg/L of benzene as the sole source of carbon, 20 mM each of electron acceptors (1740 mg/L of manganese IV oxide and 3190 mg/L of Iron III oxide), 19, 16 and 9 morphologically distinctive bacteria were isolated from soil, sediment and groundwater respectively with these characteristics. These isolates were identified using 16s rRNA region so as to exclude any potential pathogen. The microbes were further analysed to ascertain each isolate electrochemical activity capacity and biosurfactant production. The emulsification Index (EI) of biosurfactant for the respective isolates were as follows: 3 sediment isolates was 99%, 70% and 30%, 2 soil isolates was 45% and 35% and 1 groundwater isolate with 35%. For electrochemical activity, 14, 11 and 6 isolates from soil, sediment and groundwater respectively had peak current (electrochemical activity) that were greater than the negative control. On further analysis with a positive control (*Shewanella oneidensis*), 7 out of the 14 soil isolates' peak currents were greater than that of *Shewanella oneidensis* with a range of 35%-194% while 4 isolates out of the 11 sediment isolates were greater with a range of 24%-103% and 3 out of the 6 ground water isolates were greater with a range of

26%-46%. At the end of these experiments 14, 11 and 6 isolates from soil, sediment and groundwater were selected respectively.

Chapter 4 was focused on comparing MFC outputs (voltage and power density) of pure strains from each matrix and microcosm of the whole isolates from each matrix as selected in Chapter 3 above. This was carried out to ascertain which of these had better outputs so as to integrate in the next phase of MFC experiments.From the MFCs experiment were: The peak voltage output for the sediment microcosm was 270 mV groundwater microcosm while that of the pure colony was 24 mV making the microcosm peak voltage output to be 1044% greater than that of the pure colony. For the power density, the microcosm produced a peak power density of 498 (uW/m^2) while the pure colony had 53 (uW/m^2). This makes the microcosm power density to be 843% greater than the power density of the pure colony. The peak voltage output produced by the soil microcosm was 279 mV while that of the pure colony was 19 mV making the microcosm peak voltage output to be 1309% greater than that of the pure colony. The soil microcosm had a power density of 362 (uW/m^2) while the pure colony had 76 (uW/m^2) making the microcosm power density to be 374% greater than the pure colony power density. The peak voltage output for the groundwater microcosm was 286 mV groundwater microcosm while that of the pure colony was 37 mV making the microcosm peak voltage output to be 706% greater than that of the pure colony. For the peak power density, the microcosm was 222 (uW/m^2) while the pure colony was 57 (uW/m^2). This makes the microcosm power density to be 293% greater than that of the pure colony.

From the outcome of this chapter, it showed that the use of microcosm in MFCs experiments will give better outputs compared to the use of a single species. Going forward, the microcosms were used in all subsequent MFC experiments.

Chapter 5 was comparing the effects of biosurfactant (collected from the sediment isolates with 99% EI labelled A in Figure 3.16), chemical surfactant (Tween 80), the groundwater microcosm alone and a negative control in increasing the bioavailability of phenanthrene for biodegradation in a H-type MFC that mimicked groundwater matrix. The microcosm isolated from groundwater were used as inoculum.

At the end of the experiment, the MFC supplemented with the produced biosurfactant had the highest degradation rate of 280 μg/day while Tween 80 supplemented MFC had a degradation rate of 210 μg/day, microbes alone 130 μ/day and negative control 15 μg/day.

For benzene MFC that was supplemented with biosurfactant had the highest degradation rate of 850 μ/day. This was followed by the negative control with 620 μ/day while the MFCs supplemented with Tween 80 had a degradation rate of 550 μ/day the microbes alone had 370 μ/day being the least degradation rate.

Chapter 6. this Chapter focused on comparing two different types of MFC configuration (air breathing and snorkel) in sediment microbial Fuel Cell.

For this experiment, the microcosm isolated from sediment were used as inoculum while air breathing and snorkel were used as the two different Microbial Fuel Cell configurations.

The experiment had three different set ups as follows: snorkel MFC configuration, air breathing MFC configuration and natural attenuation. Air breathing MFC had the highest degradation rate 613 μg/L per day while snorkel and natural attenuation had respectively 44 μg/L/day and 1.75 μg/L/day degradation rate per day. With these results, the air breathing configuration degradation rate per day was higher than the snorkel and the natural attenuation by 92.8% and 99.7% respectively.

Chapter 7 focused on using biochar as a supplement in soil MFC.

In this experiment, the microcosm isolated from soil was used as inoculum for the MFCs. The experiment was in three different setups. MFC supplemented with biochar, MFC without biochar and natural attenuation. On the 6th day, both MFCs experiments had degraded both the phenanthrene and benzene completely to undetectable limits while the natural attenuation had a 5.8 µg/L/day and 1050 µg/L/day for phenanthrene and benzene degradation rate per day respectively.

While the soil microcosm was effective in the degradation of both phenanthrene and benzene in less than a week, the overall results show that MFC, when used, could be more efficient than natural attenuation and has great potential in the remediation of petroleum hydrocarbon across different environmental matrices

8.2 Conclusion

From the overall results of this research, it can be concluded that the integration of MFC with microcosm containing selected pure strains of microorganisms with the ability to degrade petroleum hydrocarbon, good electrochemical activity and facultative anaerobes holds great potential in the remediation of petroleum hydrocarbons pollutants. Using supplement such as biochar and biosurfactant can further enhance the degradation of petroleum hydrocarbon pollutants. A field trial and subsequent scaling up of these in petroleum polluted matrices and industrial wastes could be the most cost effective and environmentally friendly approach in remediating petroleum hydrocarbon pollutants.

8.3 Perspectives

1. The result of the microcosm that was constituted from the petroleum hydrocarbon polluted soil had the fastest degradation rate for the 200 mg of benzene and 100 mg phenanthrene in a soil MFC in less than 6 days. While the laboratory result is deemed remarkable, different experimental designs for field trial should be carried out to ascertain the most suitable set up. This is because, field is made up of different abiotic and biotic factors that play key roles in influencing the degradation of pollutants. Following the scale of the petroleum hydrocarbon pollution in the Niger-Delta as detailed by UNEP 2011, the feasible approach is an in-situ remediation. Hence, the need to carry out a field trial using different experimental designs. This could include but not limited to 1. Using different MFC configurations, 2. Constituting different microcosms by mixing at least two pure strains of the isolates to mixing the whole strains selected from each matrix. 3. Supplements like biochar and the biosurfactant that was produced by the sediment isolate that showed enhanced degradation of phenanthrene in Chapter 5 can be added. These could lead to significant improvement in the remediation of petroleum hydrocarbon in the field.

2. While the air breathing MFC configuration showed a better performance compared to the snorkel MFC configuration in Chapter 6, gradual biofouling formation was observed on the electrode (cathode) in the liquid phase at the surface of the sediment after 4 days. This may have resulted in the decline of the oxygen diffusion to the surface of the electrode that translated in the decline of the voltages transmitted that could also result in a decline in biodegradation. Biofouling of air breathing electrode has been reported to be a limitation for air breathing electrode and this may have prevented a better

degradation rate than what was recorded in Chapter 6. An MFC experiment that will investigate the effects of microbial separator as suggested by Li et. Al (2023) could enhance the performance of an air breathing MFC configuration should be carried out.

3. The results in Chapter 3 showed the ability of five isolates from sediment, soil and groundwater to produce biosurfactant or bioemulsifier. Further investigation should be carried out on the various surfactants produced by these organisms and the possible roles they could play in petroleum hydrocarbon remediation, oil recovery and their antimicrobial activity. Furthermore, a collaboration with biochemists and chemists to investigate the metabolic pathways and the intermediate metabolites that these microorganisms can produce using different substrates. Having exhibited some level of versatility in substrate uptake ranging from general medium to petroleum hydrocarbons, these microorganisms could produce additional valuable products as intermediate metabolites by utilising different substrates.

4. While the overall results of the MFC experiments showed better performance compared to the natural attenuation, the effect of adding a low voltage (<500 mV) to reactors while integrating the microcosm as inoculum on the degradation of petroleum hydrocarbon should be investigated. Such a voltage could be generated from solar panels. This may aid faster degradation of the pollutants thereby reducing the remediation period.

5. With the transitioning to renewable sources of energy for a sustainable environment and the quest to mitigate climate change, bioelectricity could be one of the key sources of renewable energy. Having discovered isolates with peak current greater than that of *S. oneidensis* with the highest being about 200% greater as recorded in Chapter 3,

investigating these isolates for bioelectricity production could play a vital role in utilising BES for large scale bioelectricity production.

6. While there is no silver bullet approach in the remediation of petroleum hydrocarbons, a combination of more than one approach could result in faster degradation and could also be more cost effective. Akunwa et al. (2014) reported sawdust as an effective biosorbent in the remediation of lead. Perhaps, the integration of BES, the microcosm and sawdust could result in the faster remediation of petroleum hydrocarbon pollutants. Hence, the need to investigate these two approaches simultaneously.

REFERENCES

Adelaja O. 2015. Bioremediation of petroleum hydrocarbons using microbial fuel cells. PhD thesis, University of Westminster.

Adelaja, O., Keshavarz, T., Kyazze, G. (2017). Treatment of phenanthrene and benzene using microbial fuel cells operated continuously for possible in situ and ex situ applications. *International Biodeterioration & Biodegradation.* 116 91-103. Available from https://search.datacite.org/works/10.1016/j.ibiod.2016.10.021 .

Adelaja, O., Keshavarz, T., Kyazze, G., (2014). Enhanced biodegradation of phenanthrene using different inoculum types in a microbial fuel cell. Eng. Life Sci. 14, 218e228. http://dx.doi.org/10.1002/elsc.201300089.

Adelana, S., Adeosun, T., (2011). Environmental pollution and remediation: challenges and management of oil spillage in the Nigerian coastal areas. *American Journal of Scientific and Industrial Research*. 2(6), 834-845. https://www.scihub.org/AJSIR/PDF/2011/6/AJSIR-2-6-834-845.pdf

Agency for Toxic Substances & Disease Registry (ATSDR), (2004). Interaction Profile for: Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX). https://www.atsdr.cdc.gov/interactionprofiles/IP-btex/ip05-p.pdf Accessed on 18th October 2020

Akankali, J. A., & Jamabo, N. A. (2012). Effects of flooding and erosion on fisheries resources in Niger Delta, Nigeria. *European Journal of Scientific Research*, 90(1), 14-25.

Akpabio, E.M., Akpan, N.S., (2010). Governance and oil politics in Nigeria's Niger Delta: the question of distributive equity. *Journal of Human Ecology* 30(2), 111–121. https://doi.org/10.1080/09709274.2010.11906280

Akunwa, N. K., Muhammad, M. N., & Akunna, J. C. (2014). *Treatment of metal-contaminated wastewater: A comparison of low-cost biosorbents*. *146*, 517–523. https://doi.org/10.1016/j.jenvman.2014.08.014

Alcantara, M.T., Gomez, J., Pazos, M., Sanroman, M.A., 2009. PAHs soil decontamination in two steps: desorption and electrochemical treatment. J. Hazard. Mater. 166, 462–468.

Ambaye, T.G., Vaccari, M., Prasad, S. & Rtimi, S. 2021, "Preparation, characterization and application of biosurfactant in various industries: A critical review on progress, challenges and perspectives", *Environmental Technology & Innovation,* vol. 24, pp. 102090.

Anifowose, B. (2008). Assessing the impacts of oil and gas transport on Nigeria's environment. In U21 Postgraduate Research Conference Proceedings 1, University of Birmingham, UK.

APHA (1999) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC. Available o

https://www.researchgate.net/profile/Arif_Reza/post/Can_you_give_me_the_APHA_method_for_trace_metal_analysis/attachment/59d63565c49f478072ea35b5/AS%3A273663767580673%401442257934916/download/standard+methods+for+the+examination+of+water+and+wastewater+%281000-3000%29.pdf

Arimoro, F.O. and Ikomi, R.B. (2009). Ecological integrity of upper Warri River, Niger Delta using aquatic insects as bioindicators. *Ecological Indicators.* 9 (3), 455-461. Available from http://www.sciencedirect.com/science/article/pii/S1470160X08000800 .

Ashfaque, F., Inam, A., Sahay, S., Iqbal, S., 2016. Influence of heavy metal toxicity on plant growth, metabolism and its alleviation by phytoremediation- A promising technology. J. Agric. Ecol. Res. Int. 6, 1–19. http://dx.doi.org/10.9734/jaeri/2016/23543.

Atlas, R.M. and Philp, J. (2005). *Bioremediation. Applied microbial solutions for real-world environmental cleanup* Washington: ASM Press.

ATSDR, (2011). Total Petroleum Hydrocarbons. Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA. https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=75 Accessed on 18 July, 2020.

Aulwar, U., Awasthi, R.S., 2016. Production of Biosurfactant and their Role in Bioremediation. J. Ecosyst. Ecogr. 6 (3), 202.

Ávila-Chávez MA, Trejo A (2010). Remediation of soils contaminated with total petroleum hydrocarbons and polycyclic aromatic hydrocarbons: Extraction with supercritical ethane. *Industrial & Engineering Chemistry Research* 49(7), 3342–3348. DOI: 10.1021/ie901502c

Azubuike C.C., Chikere C. B., Okpokwasili G. C., (2016). Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. *World J Microbiol Biotechnol* 32 (180) DOI 10.1007/s11274-016-2137-x

Bao, Y., Xu, Z., Li, Y., Yao, Z., Sun, J. & Song, H. 2017, "High-throughput metagenomic analysis of petroleum-contaminated soil microbiome reveals the versatility in xenobiotic aromatics metabolism", *Journal of Environmental Sciences,* vol. 56, pp. 25-35.

Bari, M.L. & Yeasmin, S. 2021, "Microbes Culture Methods" in *Reference Module in Biomedical Sciences. https://doi.org/10.1016/B978-0-12-818731-9.00128-2*

Beretta, G., Daghio, M., Espinoza T. A.; Franzetti, A., Mastorgio, A.F., Saponaro, S., Sezenna, E., (2019). Progress Towards Bioelectrochemical Remediation of Hexavalent Chromium. *Water 11(11),* 2336. https://doi.org/10.3390/w11112336

Besaltatpour A., Hajabbasi M., Khoshgoftarmanesh A, Dorostkar V. (2011). Landfarming process effects on biochemical properties of petroleum-contaminated soils. *Soil and Sediment Contamination: An International Journal*, 20 (2), 234-248, DOI: 10.1080/15320383.2011.546447

Biodegrad. 89, 88–94. http://dx.doi.org/10.1016/j.ibiod.2014.01.007.

Brakstad, O.G., Nordtug, T., Throne-Holst, M. (2015). Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Marine Pollution Bulletin.* 93 (1), 144-152. Available from http://www.sciencedirect.com/science/article/pii/S0025326X15000855 .

Braz. Arch. Biol. Technol. 59.

British Petroleum (BP), (2019). Statistical review of World Energy 2019. https://www.bp.com/en/global/corporate/energy-economics/statistical-review-of-worldenergy/downloads.html Accessed 11/06/2020

Cameselle, C., Gouveia, S., Urréjola, S., 2019. Benefits of phytoremediation amended with DC electric field. Application to soils contaminated with heavy metals. Chemosphere 229, 481–488, https://doi.org/10.1016/j.chemosphere.2019.04.222.

Cario, B.P., Rossi R., Kim, K., Logan, B.E. (2019). Applying the electrode potential slope method as a tool to quantitatively evaluate the performance of individual microbial electrolysis cell components. *Bioresource Technology.* 287 121418. Available: http://www.sciencedirect.com/science/article/pii/S0960852419306480 .

Chang, B.V., Shiung, L.C., Yuan S.Y. (2002) Anaerobic biodegradation of polycyclic aromatic hydrocarbon in soil. *Chemosphere,* 48:717-724.

Chen, X., Sheng, Y., Wang, G., Guo, L., Zhang, H., Zhang, F., Yang, T., Huang, D., Han, X. & Zhou, L. 2022, "Microbial compositional and functional traits of BTEX and salinity co-contaminated shallow groundwater by produced water", *Water research,* vol. 215, pp. 118277.

Chen, Y., Liu, S.A., Mou, H., Ma, Y., Li, M., Hu, X., 2017. Characterization of lipopeptide biosurfactants produced by Bacillus licheniformis MB01 from marine sediments. Front. Microbiol. 8, 871.

Cheng N, Wang B, Wu P, Lee X, Gao B. Adsorption of emerging contaminants from water and wastewater by modified biochar: a review. Environ Pollut 2021a ;273:116448.

Cheng N, Wang B, Wu P, Lee X, Xing Y, Chen M, et al. Adsorption of emerging contaminants from water and wastewater by modified biochar: a review. Environ Pollut 2021b;273:116448.

Cheng, S., Xing, D., Call, D.F., Logan, B.E., (2009). Direct biological conversion of electrical current into methane by electromethanogenesis. *Environmental Science & Technology* 43(10) 3953–3958. http://dx.doi.org/10.1021/es803531g.

Chikere CB, Chikere BO, Okpokwasili GC (2012) Bioreactor-based bioremediation of hydrocarbon-polluted Niger Delta marine sediment, Nigeria. 3 Biotech 2 53–66. doi:10.1007/s13205-011-0030-8

Chukwuka, K.S., Alimba, C.G., Ataguba, G.A. Jimoh, W.A. (2018). Chapter 9 - The Impacts of Petroleum Production on Terrestrial Fauna and Flora in the Oil-Producing Region of Nigeria. In: Ndimele, P.E., (ed.) *The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem.* Academic Press, 125-142.

Clauwaert P, Van der Ha D, Boon N, Verbeken K, Verhaege M, Rabaey K, Verstraete, W (2007) Open air biocathode enables effective electricity generation with microbial fuel cells. *Environ Sci Technol* 41(21): 7564–7569.

Clauwaert, P., Aelterman, P., Pham, H., De Schamphelaire, L. Carballa, M., Rabaey, K., Verstraete, W. (2008) Minimizing losses in bio-electrochemical systems: the road to applications. *Appl. Microbiol. Biotechnology* 79 901–913.

CNN, (2019). Major new inquiry into oil spills in Nigeria's Niger Delta launched 2019 https://edition.cnn.com/2019/03/26/africa/nigeria-oil-spill-inquiry-intl/index.html Accessed on 24/09/2020

D'Souza, G., Shitut, S., Preussger, D., Yousif, G., Waschina, S., Kost, C., 2018. Ecology and evolution of metabolic cross-feeding interactions in bacteria. Nat. Prod. Rep. 35, 455–488

Dadrasnia, A., Agamuthu, P. (2013) Diesel fuel degradation from contaminated soil by *dracaena reflexa* using organic waste supplementation. *J. the Japan Petro. Inst.*, 56(4), 236-243.

Daghio, M., Aulenta, F., Vaiopoulou, E., Franzetti, A., Arends, J.B.A., Sherry, A., Suárez-Suárez, A., Head, I.M., Bestetti, G., Rabaey, K. (2017). Electrobioremediation of oil spills. *Water Research.* 114 351-370. Available: http://www.sciencedirect.com/science/article/pii/S0043135417301185 .

Das, N., Bhuyan, B. & Pandey, P. 2022, "Correlation of soil microbiome with crude oil contamination drives detection of hydrocarbon degrading genes which are independent to quantity and type of contaminants", *Environmental research,* vol. 215, pp. 114185.

Davin, M., Starren, A., Deleu, M., Lognay, G., Colinet, G., Fauconnier, M.L., 2018. Could saponins be used to enhance bioremediation of polycyclic aromatic hydrocarbons in aged-contaminated soils? Chemosphere 194, 414–421.

Dias RL, Ruberto L, Calabro´ A, Balbo AL, Del Panno MT, Mac Cormack WP (2015) Hydrocarbon removal and bacterial community structure in on-site biostimulated biopile systems designed for bioremediation of diesel-contaminated Antarctic soil. Polar Biol 38:677–687. doi:10.1007/s00300-014-1630-7

Ebeku, K.S.A. (2004). Biodiversity conservation in Nigeria: An appraisal of the legal regime in relation to the niger delta area of the country. *Journal of Environmental Law.* 16 (3), 361-375. Available from http://www.jstor.org/stable/44248483 .

Ekeke B.A., Davies O.A., Alfred-Ockiya J.F., (2008). Sand Dredging Impact on the Fish Catch in Bonny River Estuary, Nigeria. *Environmental Research Journal*, 2(6), 299-305. https://medwelljournals.com/abstract/?doi=erj.2008.299.305

El-Hashemy, M.A. and Ali, H.M. (2018). Characterization of BTEX group of VOCs and inhalation risks in indoor microenvironments at small enterprises. *Science of the Total Environment.* 645 974-983. Available from http://www.sciencedirect.com/science/article/pii/S0048969718326470 .

Erable B, Vandecandelaere I, Faimali M, Delia M-L, Etcheverry L, Vandamme P, Bergel A (2010) Marine aerobic biofilm as biocathode catalyst. *Bioelectrochemistry* 78(1): 51–56.

Erable B., Etcheverry L., Bergel A., From microbial fuel cell (MFC) to microbial electrochemical snorkel (MES): maximizing chemical oxygen demand (COD) removal from wastewater, Biofouling 27 (2011) 319–326, https://doi.org/10.1080/08927014.2011.564615.

European Commission, (2020). Electricity driven Low Energy and Chemical input Technology foR Accelerated bioremediation (ELECTRA) https://cordis.europa.eu/project/id/826244 Accessed on 22nd July, 2020.

Evans, M.G., Furlong, C.J. (2011) *Environmental biotechnology theory and application*. John Wiley & Sons, Inc. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9780470975152.fmatter

Ewing T, Phuc Thi H, Beyenal H. Evaluation of long-term performance of sediment microbial fuel cells and the role of natural resources. Appl Energy 2017;192:490e7.

F. Aulenta, M. Tucci, C. Cruz Viggi, J. Dolfing, I.M. Head, A.E. Rotaru, An underappreciated DIET for anaerobic petroleum hydrocarbon-degrading microbial communities, Microb. Biotechnol. 14 (2021) 2–7, https://doi.org/ 10.1111/1751-7915.13654.

Fan, Y., Sharbrough, E., Liu, H., (2008) Quantification of the Internal Resistance Distribution of Microbial Fuel Cells. *Environ. Sci. Technol*., 42:8101-8107.

Felix, A.K.N., Martins, J.J., Almeida, J.G.L., Giro, M.E.A., Cavalcante, K.F., Melo, V.M.M.,Pessoa, O.D.L., Rocha, M.V.P., Gonçalves, L.R.B., de Santiago Aguiar, R.S., 2019. Purification and characterization of a biosurfactant produced by Bacillus subtilis in cashew apple juice and its application in the remediation of oil-contaminated soil. Colloids Surf. B 175, 256–263.

Franks A. E.,Nevin K. P (2010) Microbial Fuel Cells, A Current Review. *Energies* 3:899-919.

Frick, C. M., Farrell, R. E., Germida, J. J. (1999). *Assessment of Phytoremediation as an in-situ technique for cleaning oil-contaminated sites*. https://clu-in.org/download/remed/phyassess.pdf Accessed on 6th March 2020.

Friman, H., Schechter, A., Nitzan, Y., Cahan, R., 2012. Effect of external voltage on Pseudomonas putida F1 in a bioelectrochemical cell using toluene as sole carbon and energy source. *Microbiology* 158, 414e423. http://dx.doi.org/10.1099/mic.0.053298-0.

Frutos FJG, Pe´rez R, Escolano O, Rubio A, Gimeno A, Fernandez MD, Carbonell G, Perucha C, Laguna J (2012) Remediation trials for hydrocarbon-contaminated sludge from a soil washing process: evaluation of bioremediation technologies. J Hazard Mater 199:262–271. doi:10.1016/j.jhazmat.2011.11.017

Fuentes, S., Méndez, V., Aguila, P., Seeger, M., 2014. Bioremediation of petroleum hydrocarbons: catabolic genes, microbial communities, and applications.Appl. Microbiol. Biot. 98, 4781–4794.

Gao, H., Wu, M., Liu, H., Xu, Y. & Liu, Z. 2022, "Effect of petroleum hydrocarbon pollution levels on the soil microecosystem and ecological function", *Environmental Pollution,* vol. 293, pp. 118511.

Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y. (2016). Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Frontiers in Microbiology*. 7 1369. Available from https://search.datacite.org/works/10.3389/fmicb.2016.01369 .

Gomez, F. and Sartaj, M. (2014). Optimization of field scale biopiles for bioremediation of petroleum hydrocarbon contaminated soil at low temperature conditions by response surface methodology (RSM). *International Biodeterioration & Biodegradation.* 89 103-109. Available from http://www.sciencedirect.com/science/article/pii/S0964830514000146 .

Gregory, K.B., Lovley, D.R., (2005). Remediation and recovery of uranium from contaminated subsurface environments with electrodes. *Environmental Science & Technology* 39(22), 8943–8947. http://dx.doi.org/10.1021/es050457e.

Guo, H.Y., Tang, S.F., Xie, S.X., Wang, P.H., Huang, C.F., Geng, X.H., Jia, X.L., Huo, H.J., Li, X.P., Zhang, J.Q., Zhang, Z.W., Fang, J.D., 2020. The oil removal and the characteristics of changes in the composition of bacteria based on the oily sludge bioelectrochemical system. Sci. Rep. 10, 15474. https://doi.org/10.1038/s41598- 020-72405-9.

H. Liu, B.E. Logan, Environ. Sci. Technol. 38 (2004) 4040e4046.

H. Liu, S. Cheng, B.E. Logan, Environ. Sci. Technol. 40 (2006) 2426e2432.

Haritash, A.K., Kaushik, C.P. (2009) Biodegradation Aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *J. Hazard. Mater.,* 169 (1-3):1-15.

Hernández, M.A., Ojeda-Morales, M.E., Martínez–Vázquez, J.G., Villegas-Cornelio, V.M., Córdova-Bautista, Y. (2011) Optimal Parameters For In Vitro Development of the Hydrocarbonoclastic Microorganism *Proteus sp. J. Soil Sci. Plant Nutr*.11(1):29 – 43.

Hu, W.-J., Niu, C.-G., Wang, Y., Zeng, G.-M., Wu, Z. (2011) Nitrogenous heterocyclic compounds degradation in the microbial fuel cells. *Process Safety and Environ. Prot., 89*(2):133-140.

Huang, L., Chai, X., Chen, G., Logan, B.E., (2011). Effect of set potential on hexavalent chromium reduction and electricity generation from biocathode microbial fuel cells. *Environmental Science & Technology* 45(11), 5025–5031. http://dx.doi.org/10.1021/es103875d.

HYDROCARBON pollution remediation project (HYPREP), (2020). Remediation work. https://hyprep.gov.ng/remediation-work/ Accessed on 5 October 2020

International Tanker Owners Pollution Federation (**ITOPF**), (2019). Oil tanker spills statistics 2018.http://www.itopf.org/fileadmin/data/Documents/Company_Lit/Oil_Spill_Stats_2018.pdf Accessed on 27th May 2020

Islam, M.A., Ethiraj, B., Cheng, C.K., Yousuf, A., Khan, M.M.R., 2018a. An insight of synergy between Pseudomonas aeruginosa and Klebsiella variicola in a microbial fuel cell. ACS Sustain. Chem. Eng. 6, 4130–4137.

Ite, A.E., Ibok, U.J., Ite, M.U., Petters, S.W., (2013). Petroleum exploration and production: past and present environmental issues in the Nigeria's Niger Delta. *American Journal of Environmental Protection* 1(4), 78–90.

Juliana E. B., Ergas, S.J., Chang, D.P.Y., Schroeder, E.D. (1998). *Bioremediation principles* Maidenhead: McGraw-Hill Book Company Europe.

Kadafa A. A., (2012). Environmental Impacts of Oil Exploration and Exploitation in the Niger Delta of Nigeria. *Global Journal of Science Frontier Research Environment & Earth Sciences, 12 (3), 19-28. https://globaljournals.org/GJSFR_Volume12/2-Environmental-Impacts-of-Oil-Exploration.pdf*

Kaskatepe, B., Yildiz, S., 2016. Rhamnolipid biosurfactants produced by Pseudomonas species.

Kavitha, V., Mandal, A.B., Gnanamani, A., 2014. Microbial biosurfactant mediated removal and/or solubilization of crude oil contamination from soil and aqueous phase: an approach with Bacillus licheniformis MTCC 5514. Int. Biodeterior. Biodegrad. 94, 24-30.

Khan, F.I., Husain, T., Hejazi, R. (2004). An overview and analysis of site remediation technologies. *Journal of Environmental Management.* 71 (2), 95-122. Available from http://www.sciencedirect.com/science/article/pii/S0301479704000313 .

Könnet, B.R., (2014). Inadequate monitoring and enforcement in the Nigerian oil industry: The case of shell and Ogoniland. Cornell Int. Law J. 11, 181–205. https://heinonline.org/HOL/LandingPage?handle=hein.journals/cintl47&div=9&id=&page=

Korlević, M., Zucko, J., Dragić, M.N., Blažina, M., Pustijanac, E., Zeljko, T.V., Gacesa, R., Baranasic, D., Starcevic, A., Diminic, J., Long, P.F., Cullum, J., Hranueli, D. & Orlić, S. 2015, "Bacterial diversity of polluted surface sediments in the northern Adriatic Sea", *Systematic and applied microbiology,* vol. 38, no. 3, pp. 189-197.

Kuiper, I., Lagendijk, E.L., Bloemberge, G.V., lugtenberg, B.I. (2004) Rhizoremediation: A beneficial plant - microbe interaction, *Molecular Plant-Microbe Interactions*, 17 6-15. https://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI.2004.17.1.6

Lai, C.C., Huang, Y.C.,Wei, Y.H., Chang, J.S., 2009. Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. J. Hazard. Mater. 167, 609–614.

Latif, M.T., Abd Hamid, H.H., Ahamad, F., Khan, M.F., Mohd Nadzir, M.S., Othman, M., Sahani, M., Abdul Wahab, M.I., Mohamad, N., Uning, R., Poh, S.C., Fadzil, M.F., Sentian, J., Tahir, N.M. (2019). BTEX compositions and its potential health impacts in Malaysia. *Chemosphere (Oxford).* 237 124451. Available from https://search.datacite.org/works/10.1016/j.chemosphere.2019.124451 .

Li, C., Yi, K., Hu, S., Yang, W., 2023. Cathodic biofouling control by microbial separators in air-breathing microbial fuel cells. Environmental Science and Ecotechnology 15, 100251. https://doi.org/10.1016/j.ese.2023.100251

Li, T., Li, R., Zhou, Q. (2020). The application and progress of bioelectrochemical systems (BESs) in soil remediation: A review. *Green Energy & Environment.* Available from http://dx.doi.org/10.1016/j.gee.2020.06.026 .

Li, Y.-M., Haddad, N.I., Yang, S.-Z., Mu, B.-Z., 2008. Variants of lipopeptides produced by Bacillus licheniformis HSN221 in different medium components evaluated by a rapid method ESI-MS. Int. J. Pept. Res. Ther. 14, 229–235.

Liang, B., Ma, J., Cai, W., Li, Z., Liu, W., Qi, M., Zhao, Y., Ma, X., Deng, Y., Wang, A., Zhou, J. (2019). Response of chloramphenicol-reducing biocathode resistome to continuous electrical stimulation. *Water Research.* 148 398-406. Available from http://www.sciencedirect.com/science/article/pii/S0043135418308765 .

Lim MW, Von Lau E, Poh PE (2016) A comprehensive guide of remediation technologies for oil contaminated soil–present works and future directions. *Marine pollution bulletin* 109(1): 14–45.

Liu, C., Shi, W., Li, H., Lei, Z., He, L., Zhang, Z. (2014). Improvement of methane production from waste activated sludge by on-site photocatalytic pretreatment in a photocatalytic anaerobic fermenter. *Bioresource Technology.* 155 198-203. Available from http://www.sciencedirect.com/science/article/pii/S0960852413018701

Liu, L., Li, W., Song, W., Guo, M., 2018. Remediation techniques for heavy metal-contaminated soils: Principles and applicability. Sci. Total Environ.633, 206–219. http://dx.doi.org/10.1016/j.scitotenv.2018.03.161.

Liu, W., Cheng, S., Sun, D., Huang, H., Chen, J., Cen, K., 2015. Inhibition of microbial growth on air cathodes of single chamber microbial fuel cells by incorporating enrofloxacin into the catalyst layer. Biosens. Bioelectron. 72, 44–50. https://doi. org/10.1016/j.bios.2015.04.082.

Logan BE (2009) Exoelectrogenic bacteria that power microbial fuel cells. *Nature Reviews Microbiology* 7 375–381. https://www.nature.com/articles/nrmicro2113

Logan, B.E. (2008) *Microbial Fuel cells*. John Wiley and Sons, New Jersey.

Logan, B.E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. (2006) Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.,* 40:5181-5192.

Logan, B.E., Rossi, R., Saikaly, P.E., 2019. Electroactive microorganisms in bioelectrochemical systems. Nat. Rev. Microbiol. 17, 307–319.

Lovley DR (2006) Bug juice: harvesting electricity with microorganisms. *Nat Rev Microbiol* 4(7): 497

Lu, L., Yazdi, H., Jin, S., Zuo, Y., Fallgren, P.H., Ren, Z.J. (2014). Enhanced bioremediation of hydrocarbon-contaminated soil using pilot-scale bioelectrochemical systems. *Journal of Hazardous Materials.* 274 8-15. Available from http://www.sciencedirect.com/science/article/pii/S0304389414002465 .

Lundstedt, S. (2003). Analysis of PAHs and their transformations products in contaminated soil and remedial processes (PhD dissertation). Kemi, Umeå. Retrieved from http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-57

Lusk B.G., 2019: Thermophiles; or, the modern Prometheus: the importance of extreme microorganisms for understanding and applying extracellular electron transfer. Front Microbiol, 10:818.

Ma, Y., Zhao, H., Shan, Q., Xu, Y., Yu, M., Cui, J., Liu, T., Qiao, L. & He, X. 2021, "K-strategy species plays a pivotal role in the natural attenuation of petroleum hydrocarbon pollution in aquifers", *Journal of hazardous materials,* vol. 420, pp. 126559.

Maila M.P., Colete TE (2004) Bioremediation of petroleum hydrocarbons through land farming: are simplicity and cost-effectiveness the only advantages? Rev Environ Sci Bio/Technol 3 349–360. doi: 10.1007/s111157-004-6653

Mandal, A., Purakayastha, T.J., Ramana, S., Neenu, S., Bhaduri, D., Chakraborty, K., Manna, M.C., Rao, A.S., 2014. Status on phytoremediation of heavymetals in India- A review. Int. J. Bio-Resour. Stress Manag. 5, 553. http://dx.doi.org/10.5958/0976-4038.2014.00609.5.

Marć, M., Bielawska, M., Simeonov, V., Namieśnik, J., Zabiegała, B. (2016). The effect of anthropogenic activity on BTEX, NO2, SO2, and CO concentrations in urban air of the spa city of Sopot and medium-industrialized city of Tczew located in North Poland. *Environmental Research.* 147 513-524. Available from http://www.sciencedirect.com/science/article/pii/S0013935116300949 .

Martinez, C.M. and Alvarez, L.H. (2018). Application of redox mediators in bioelectrochemical systems. *Biotechnology Advances.* 36 (5), 1412-1423. Available from https://search.datacite.org/works/10.1016/j.biotechadv.2018.05.005 .

Matturro, B., Viggi, C. C., Aulenta, F., Rossetti, S., Cable bacteria and the bioelectrochemical snorkel: the natural and engineered facets playing a role in hydrocarbons degradation in marine sediments, Front. Microbiol. 8 (2017) 952, , https://doi.org/10.3389/fmicb.2017.00952.

Michel, J., & Fingas, M. (2016). Oil spills: Causes, consequences, prevention, and counter measures. *Fossil Fuels* 159-201. https://doi.org/10.1142/9789814699983_0007

Milic, J.S., Beskoski, V.P., Ilic, M.V., Ali S.A.M., Gojgic-Cvijovic, G.D., Vrvic, M.M. (2009) Bioremediation of soil heavily contaminated with crude oil and its products: Composition of the microbial consortium. *J. Serbian Chem. Soc*., 74:455-460.

Mishra, S., Lin, Z., Pang, S., Zhang, W., Bhatt, P., Chen, S., 2021b. Recent advanced technologies for the characterization of xenobiotic-degrading microorganisms and microbial communities. Front. Bioeng. Biotechnol. https://doi.org/10.3389/fbioe.2021.632059.

Mohan, S.V., Sirisha, K., Rao, N.C., Sarma, P.N., Reddy, S.J. (2004). Degradation of chlorpyrifos contaminated soil by bioslurry reactor operated in sequencing batch mode: bioprocess monitoring. *Journal of Hazardous Materials.* 116 (1), 39-48. Available from http://www.sciencedirect.com/science/article/pii/S0304389404003462 .

Morgane H, Benjamin E., Alain B. 2019 Microbial electrochemical snorkels (MESs): A budding technology for multiple applications. A mini review, *Electrochemistry Communications*, Vol. 104, Pg 2

Morris JM, Jin S (2012) Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells. *J Hazard Mater* 213: 474–477.

Morris JM, Jin S. Enhanced biodegradation of hydrocarboncontaminated sediments using microbial fuel cells. J Hazard Mater 2012;213:474e7.

Morris, J. M., Jin, S. (2012). Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells. *J. Hazard. Mater.,* 213–214(0):474-477.

Mustafa YA, Abdul-Hameed HM, Razak ZA (2015) Biodegradation of 2,4-dichlorophenoxyacetic acid contaminated soil in a roller slurry bioreactor. Clean-Soil Air Water 43 (8), 1115–1266. https://doi.org/10.1002/clen.201400623

Myers C. R. and Nealson K. H., (1988). Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor. *Science* 4857 (240): 1319-1321 DOI: 10.1126/science.240.4857.c1319

National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, (2011). The use of surface and subsea dispersants during the BP Deepwater Horizon Oil Spill https://www.washingtonpost.com/wp-srv/nation/pdfs/ResponseRDWorkingPaper.pdf Accessed on 31st July 2020.

National Oil Spill Detection and Response Agency (NOSDRA), (2020). Data Summary https://oilspillmonitor.ng

Ndimele, P.E., Akanni, A., Shittu, J.A., Ewenla, L.O. Ige, O.E. (2018). Chapter 16 - Trade-off Analyses of Ecosystem Services in Nigerian Waters. In: Ndimele, P.E., (ed.) *The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem.* Academic Press, 229-256

Nikolopoulou, M., Pasadakis, N., Norf, H., Kalogerakis, N. (2013). Enhanced ex situ bioremediation of crude oil contaminated beach sand by supplementation with nutrients and rhamnolipids. *Marine Pollution Bulletin.* 77 (1), 37-44. Available from http://www.sciencedirect.com/science/article/pii/S0025326X13006590 .

Nikolova, C and Gutierrez, T. 2021: Biosurfactants and Their Applications in the Oil and GasIndustry: Current State of Knowledge and Future Perspectives. Frontiers in Bioengineering and Biotechnology, 626639:272-290

Nwilo, P.C., Badejo, O.T., (2006). Impacts and management of oil spill pollution along the Nigerian coastal areas. *Administering Marine Spaces: International issues.* 119. https://www.digi.fig.net/resources/publications/figpub/pub36/pub36.pdf#page=121

O'Connor, D., Hou, D., Ok, Y.S., Song, Y., Sarmah, A.K., Li, X., et al., 2018. Sustainable in situ remediation of recalcitrant organic pollutants in groundwaterwith controlled release materials: a review. J. Control. Release 283, 200–213.

Oh, S. and Logan, B.E. (2005). Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. *Water Research.* 39 (19), 4673-4682. Available from http://www.sciencedirect.com/science/article/pii/S0043135405005129 .

Okotie, S., Ogbarode, N.O. Ikporo, B. (2018). Chapter 4 - The Oil and Gas Industry and the Nigerian Environment. In: Ndimele, P.E., (ed.) *The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem.* Academic Press, 47-69.

Olarinmoye, O.M., Ugwumba, O.A. Awe, F.O. (2018). Chapter 1 - The Nigerian Environment. In: Ndimele, P.E., (ed.) *The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem.* Academic Press, 3-15. https://doi.org/10.1016/B978-0-12-809399-3.00001-X

Onuoha, F. C. (2008). Oil pipeline sabotage in Nigeria: Dimensions, actors and implications for national security. *African Security Review* 17(3), 99-115. https://doi.org/10.1080/10246029.2008.9627487

OPEC (2024). 2024 World oil outlook 2050. https://publications.opec.org/woo. Accessed on 24/11/2024

Organization of the Petroleum Exporting Countries (OPEC), (2015). Nigeria: Facts and Figures. URL. http://www.opec.org/opec_web/en/about_us/167.htm.

Organization of the Petroleum Exporting Countries (OPEC), (2019). OPEC Statement to the UN Climate Change Conference (COP 25/CMP 15/CMA 2) https://www.opec.org/opec_web/en/press_room/5809.htm accessed on 6/6/2020

Pankratova G, Hederstedt L, Gorton L., 2019: Extracellular electron transfer features of Gram-positive bacteria. Anal Chim Acta 1076:32–47..

Pant, V. B, Gilbert, D. S, Mark, D., Ludo, V., Karolien, T. (2010) Use of novelpermeable membrane and air cathodes in acetate microbial fuel cells. *Electrochimica Acta.* 55(26), 7710-7716.

Pegg, S., Zabbey, N., (2013). Oil and water: the Bodo spills and the destruction of traditional livelihood structures in the Niger Delta. Community Development Journal 48(3), 391–405. http://dx.doi.org/10.1093/cdj/bst021

Pei, G., Zhu, Y., Cai, X., Shi, W., Li, H., 2017. Surfactant flushing remediation of odichlorobenzene and p-dichlorobenzene contaminated soil. Chemosphere 185, 1112–1121.

Phulpoto, I.A., Hu, B., Wang, Y., Ndayisenga, F., Li, J. & Yu, Z. 2021, "Effect of natural microbiome and culturable biosurfactants-producing bacterial consortia of freshwater lake on petroleum-hydrocarbon degradation", *Science of The Total Environment,* vol. 751, pp. 141720.

Pouresmaeili, M.A., Aghayan, I., Taghizadeh, S.A. (2018). Development of Mashhad driving cycle for passenger car to model vehicle exhaust emissions calibrated using on-board measurements. *Sustainable Cities and Society.* 36 12-20. Available from http://www.sciencedirect.com/science/article/pii/S221067071730728X .

Rajkumar, M., Sandhya, S., Prasad, M.N.V., Freitas, H., 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol.Adv. 30, 1562–1574. http://dx.doi.org/10.1016/j.biotechadv.2012.04.011.

Ran, J., Qiu, H., Sun, S., Tian, L. (2018). Short-term effects of ambient benzene and TEX (toluene, ethylbenzene, and xylene combined) on cardiorespiratory mortality in Hong Kong. *Environment International.* 117 91-98. Available from http://www.sciencedirect.com/science/article/pii/S0160412018300126 .

Rico-Martínez, R., Terry, W. S., Tonya, L. S. (2013) Synergistic toxicity of Macondo crude oil and dispersant to the *Brachionus plicatilis* species complex (Rotifera) *Environ. Poll.*,173:5–10.

Rosenbaum, M.A., Franks, A.E (2014) Microbial catalysis in bioelectrochemical technologies: status quo, challenges and perspectives. *Appl. Microbiol. Biotechnol*ogy 98 509–518.

S. Goel, M. Munjal, R.K. Sharma, G. Singh, Woodhead Publishing (2021) 339-371. https://doi.org/10.1016/B978-0-12-820484-9.00014-3.

Sajna, K.V., Sukumaran, R.K., Gottumukkala, L.D., Pandey, A., (2015). Crude oil biodegradation aided by biosurfactants from Pseudozyma sp. NII 08165 or its culture broth. Bioresource Technol. 191, 133–139

Sajna, K.V., Sukumaran, R.K., Gottumukkala, L.D., Pandey, A., (2015). Crude oil biodegradation aided by biosurfactants from Pseudozyma sp. NII 08165 or its culture broth. Bioresource Technol. 191, 133–139

Salau, A.T. (1993). Environmental Crisis and Development in Nigeria. Inaugural Lecture, delivered on Thursday, 11th February, 1993 at the University of Port Harcourt, Nigeria. Inaugural Lectures Series No. 13, 38 .https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.460.7133&rep=rep1&type=pdf Accessed on 6th June 2020

Sanscartier, D., Zeeb, B., Koch, I., Reimer, K. (2009). Bioremediation of diesel-contaminated soil by heated and humidified biopile system in cold climates. *Cold Regions Science and Technology.* 55 (1), 167-173. Available from http://www.sciencedirect.com/science/article/pii/S0165232X08001080 .

Santoro C., Arbizzani C., Erable B., Ieropoulos I., Microbial fuel cells: from fundamentals to applications. A review, J. Power Sources 356 (2017) 225–244, https://doi.org/10.1016/j.jpowsour.2017.03.109.

Serra PMD, Espirito-Santo A. Sourcing power with microbial fuel cells: a timeline. J Power Sources 2021:482.

Shen, J., Xu, X., Jiang, X., Hua, C., Zhang, L., Sun, X., Li, J., Mu, Y., Wang, L. (2014). Coupling of a bioelectrochemical system for p-nitrophenol removal in an upflow anaerobic sludge blanket reactor. *Water Research.* 67 11-18. Available from http://www.sciencedirect.com/science/article/pii/S0043135414006319 .

Siegrist RL, Crimi M, Simpkin TJ (2011) *In situ Chemical Oxidation for Groundwater Remediation*. Springer Science & Business Media. New York.

Sleutels, T.A., Libertus, D., Hubertus, V.M., Cees, J.N. (2011) Effect of operational parameters on Coulombic efficiency in bioelectrochemical systems. *Bioresour. Technol*., 102(24):11172-11176.

Smith E, Thavamani P, Ramadass K, Naidu R, Srivastava P, Megharaj M (2015) Remediation trials for hydrocarbon-contaminated soils in arid environments: evaluation of bioslurry and biopiling techniques. Int Biodeterior Biodegradation 101 56–65. doi:10.1016/j.ibiod.2015.03.029

Souza, E.C., Vessoni-Penna, T.C., de Souza Oliveira, R.P., 2014. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. Int. Biodeterior.

Spalding,M., Kainuma, M., Collins, L., (2010). World Atlas of Mangroves. Earthscan, London. https://books.google.co.uk/books?hl=en&lr=&id=Mm6O0ab7uaMC&oi=fnd&pg=PR1&dq=+73.%09Spalding,M.,+Kainuma,+M.,+Collins,+L.,+2010.+World+Atlas+of+Mangroves.+Earthscan,+London.&ots=1LqPFHzp4k&sig=IEC5caQrj4UKutojFggpUyM4AVo#v=onepage&q&f=false

Speight, J.G. (2018). Chapter 8 - Biological Transformations. In: Speight, J.G., (ed.) *Reaction Mechanisms in Environmental Engineering.* Butterworth-Heinemann, 269-306.

Srikanth, S., Kumar, M., Puri, S.K. (2018). Bio-electrochemical system (BES) as an innovative approach for sustainable waste management in petroleum industry. *Bioresource Technology.* 265 506-518. Available from http://www.sciencedirect.com/science/article/pii/S0960852418302529 .

Srikanth, S., Kumar, M., Singh, D., Singh, M.P., Das, B.P. (2016). Electro-biocatalytic treatment of petroleum refinery wastewater using microbial fuel cell (MFC) in continuous mode operation. *Bioresource Technology.* 221 70-77. Available from http://www.sciencedirect.com/science/article/pii/S0960852416312895 .

Suthersan, Suthan, S., Payne, F. C. (2005) *In Situ* Remediation Engineering. Boca Raton, FL: CRC Press. Taylor & Francis Group.

Taccari, M., Milanovic, V., Comitini, F., Casucci, C., Ciani, M. (2012). Effects of biostimulation and bioaugmentation on diesel removal and bacterial community. *International Biodeterioration & Biodegradation.* 66 (1), 39-46. Available from http://www.sciencedirect.com/science/article/pii/S0964830511002125 .

Talley JW, Sleeper PM. Roadblocks to the implementation of biotreatment strategies (1997). Ann N Y Acad Sci. 829 16-29. DOI: 10.1111/j.1749-6632.1997.tb48562.x

Tewari, S., & Sirvaiya, S. (2015). Oil spill remediation and its regulation. International Journal of Research in Science & Engineering, 1(6), 1-7.

Tian., 2019; Tian T, Fan X, Feng M, Su L, Zhang W, Chi H, Fu D: Flavin mediated extracellular electron transfer in Gram-positive bacteria Bacillus cereus DIF1 and Rhodococcus ruber DIF2. RSC Adv 2019, 9:40903–40909.

U.S. Energy Information Administration (EIA) (2019). International Energy Outlook 2019 https://www.eia.gov/outlooks/ieo/pdf/ieo2019.pdf Accessed on 5th June 2020

United Nations Development Programme (UNDP). (2016). Niger Delta human development report. Abuja: United Nations Development Programme. Abuja: United Nations Development Programme, 229. http://hdr.undp.aorg/sites/default/files/nigeria_hdr_report.pdf. Accessed on 6th June 2020

United Nations Environment Programme UNEP, (2011) Environmental Assessment of Ogoniland [Online] Available at: http://postconflict.unep.ch/publications/OEA/UNEP_OEA.pdf Accessed on 5/2/2020

United States Environmental protection Agency (USEPA), 2017. Dispersing Agents https://www.epa.gov/emergency-response/dispersing-agents Accessed on 6th March 2020

Usman, M., Faure, P., Ruby, C., Hanna, K. (2012). Remediation of PAH-contaminated soils by magnetite catalyzed Fenton-like oxidation. *Applied Catalysis B: Environmental.* 117-118 10-17. Available from http://www.sciencedirect.com/science/article/pii/S0926337312000082 .

Van Hamme, J.D., Singh, A., Ward, O.P., 2003. Recent advances in petroleum microbiology. Microbiol. Mol. Biol. Rev. 67, 503–549. https://doi.org/10.1128/ MMBR.67.4.503-549.2003.

Varjani, S.J., 2017. Microbial degradation of petroleum hydrocarbons. Bioresource Technol. 223, 277–286.

Varjani, S.J., 2017. Microbial degradation of petroleum hydrocarbons. Bioresource Technol. 223, 277–286.

Varjani, S.J., Upasani, V.N., 2016. Core flood study for enhanced oil recovery through ex-situ bioaugmentation with thermo- and halo-tolerant rhamnolipid produced by Pseudomonas aeruginosa NCIM 5514. Bioresour. Technol. 220

Verma JP, Jaiswal DK (2016) Book review: advances in biodegradation and bioremediation of industrial waste. Front Microbiol 6 1–2. doi:10.3389/fmicb.2015.01555

Viggi C. C., Presta E., Bellagamba M., Kaciulis S., Balijepalli S.K., Zanaroli G., Papini M. P., Rossetti S., Aulenta F., The "Oil-Spill Snorkel": an innovative bioelectrochemical approach to accelerate hydrocarbons biodegradation in marine sediments, Front. Microbiol. 6 (2015) 881, https://doi.org/10.3389/fmicb.2015.00881.

Wang H., Lu L., Ren Z. J. (2020). Enhanced bioremediation of petroleum hydrocarbons using microbial electrochemical technology. CRC Press: Taylor & Francis Group.

Wang H., Lu L., Ren Z. J. (2020a). Enhanced Bioremediation of Petroleum Hydrocarbons Using Microbial Electrochemical Technology, In: Tiquia-Arashiro S. and Pant, D. (Editors), Microbial Electrochemical Technologies, CRC Press, 343-359.

Wang X, Cai Z, Zhou Q, Zhang Z, Chen C (2012) Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. *Biotechnol Bioeng* 109(2):426–433.

Wang X., Aulenta F., Puig S., Esteve-Nú˜nez A., He Y., Mu Y., Rabaey K., 2020. Microbial electrochemistry for bioremediation. Environmental Science and Ecotoxicology. 1: 100013

Wang, H., Luo, H., Fallgren, P.H., Jin, S., Ren, Z.J. (2015). Bioelectrochemical system platform for sustainable environmental remediation and energy generation. *Biotechnology Advances.* 33 (3), 317-334. Available from http://www.sciencedirect.com/science/article/pii/S0734975015000683 .

Wang, V.B., Chua, S.-L., Cai, Z., Sivakumar, K., Zhang, Q., Kjelleberg, S., et al., 2014. A stablesynergistic microbial consortium for simultaneous azo dye removal and bioelectricitygeneration. Bioresour. Technol. 155, 71–76.

Wang, V.B., Sivakumar, K., Yang, L., Zhang, Q., Kjelleberg, S., Loo, S.C.J., et al., 2015.Metabolite-enabled mutualistic interaction between Shewanella oneidensis andEscherichia coli in a co-culture using an electrode as electron acceptor. Sci. Rep. 5,1–11.

Wang, V.B., Sivakumar, K., Yang, L., Zhang, Q., Kjelleberg, S., Loo, S.C.J., et al., 2015.Metabolite-enabled mutualistic interaction between Shewanella oneidensis and Escherichia coli in a co-culture using an electrode as electron acceptor. Sci. Rep. 5,1–11.

Wang, X., Cai, Z., Zhou, Q., Zhang, Z., Chen, C. (2012b) Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. *Biotech. and Bioeng.,* 109:426-433.

Wang, X., Cai, Z., Zhou, Q.X., Zhang, Z.N., Chen, C.H., 2012. Bioelectrochemical stimulation of petroleum hydrocarbon degradation in saline soil using U-tube microbial fuel cells. *Biotechnology and Bioengineering* 109 (2), 426–433.

Wani, A.K., Akhtar, N., Naqash, N., Chopra, C., Singh, R., Kumar, V., Kumar, S., Mulla, S.I. & Américo-Pinheiro, J.H.P. 2022, "Bioprospecting culturable and unculturable microbial consortia through metagenomics for bioremediation", Cleaner Chemical Engineering, vol. 2, pp. 100017.

Wartell, B., Boufadel, M., Rodriguez-Freire, L., 2021. An effort to understand and improve the anaerobic biodegradation of petroleum hydrocarbons: a literature review. Int. Biodeterior. Biodegrad. 157, 105156. https://doi.org/10.1016/j. ibiod.2020.105156.

Wei, M., Rakoczy, J., Vogt, C., Harnisch, F., Schumann, R., Richnow, H.H. (2015). Enhancement and monitoring of pollutant removal in a constructed wetland by microbial electrochemical technology. *Bioresource Technology.* 196 490-499. Available from http://www.sciencedirect.com/science/article/pii/S0960852415010822 .

Wei, W., Ran, Z., He, H., Zhou, K., Huangfu, Z., Yu, J., 2020. Desorption process andmorphological analysis of real polycyclic aromatic hydrocarbons contaminated soil by theheterogemini surfactant and its mixed systems. Chemosphere 254, 126854.

Wen-xiang, X., LI, J., SONG, Z., SUN, Y. (2007). Effects of nitrate concentration in interstitial water on the bioremediation of simulated oil-polluted shorelines. *Journal of Environmental Sciences.* 19 (12), 1491-1495. Available from http://www.sciencedirect.com/science/article/pii/S1001074207602423 .

Whelan, M.J., Coulon, F., Hince, G., Rayner, J., McWatters, R., Spedding, T., Snape, I. (2015). Fate and transport of petroleum hydrocarbons in engineered biopiles in polar regions. *Chemosphere.* 131 232-240. Available from http://www.sciencedirect.com/science/article/pii/S0045653514014453 .

World Energy Council. 2019. World Energy Trilemma Index 2019 https://www.worldenergy.org/publications/entry/world-energy-trilemma-index-2019 Accessed on 17th May, 2020.

World Health Organisation (WHO), 2000. Air Quality Guidelines - Second Edition https://www.euro.who.int/ data/assets/pdf file/0017/123056/AQG2ndEd 5 2benzene.pdf Accessed on 19/10/20

Wu, Y., Jing, X., Gao, C., Huang, Q., Cai, P. (2018). Recent advances in microbial electrochemical system for soil bioremediation. *Chemosphere (Oxford).* 211 156-163. Available: https://search.datacite.org/works/10.1016/j.chemosphere.2018.07.089 .

Xiang, Y., Liu, G., Zhang, R., Lu, Y., Luo, H. (2017). High-efficient acetate production from carbon dioxide using a bioanode microbial electrosynthesis system with bipolar membrane. *Bioresource Technology.* 233 227-235. Available from http://www.sciencedirect.com/science/article/pii/S096085241730233X .

Xiao, X. & Yu, H. 2020, "Molecular mechanisms of microbial transmembrane electron transfer of electro active bacteria", *Current opinion in chemical biology,* vol. 59, pp. 104-110.

Xu et al., 2018. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. Frontiers in microbiology. December (2018): 1-11.

Xu L, Zhang H, Xiong P, Zhu Q, Jiang G. Occurrence, fate, and risk assessment of typical tetracycline antibiotics in the aquatic environment: a review. Sci Total Environ 2020;753:141975.

Yan Z, He Y, Cai H, Van Nostrand JD, He Z, Zhou J, et al. Interconnection of key microbial functional genes for enhanced benzo[a]pyrene biodegradation in sediments by microbial electrochemistry. Environ Sci Technol 2017;51:8519e29.

You LX, Liu LD, Xiao Y, Dai YF, Chen BL, Jiang YX, Zhao F. 2018: Flavins mediate extracellular electron transfer in Grampositive Bacillus megaterium strain LLD-1. Bioelectrochemistry, 119:196–202.

Zabbey, N., 2009. Impacts of oil pollution on livelihoods in Nigeria. Petroleum and Pollution— How Does That Impact Human Rights? Amnesty International, Forum Syd and Friends of the Earth, Sweden Kulturhuset, Stockholm, Sweden. (27th April 2009). https://nairametrics.com/wp-content/uploads/2013/01/impact-of-Oil_and_livelihoods2.pdf

Zabbey, N., Sam, K., Onyebuchi, A.T. (2017). Remediation of contaminated lands in the Niger Delta, Nigeria: Prospects and challenges. *The Science of the Total Environment.* 586 952-965. Available from https://search.datacite.org/works/10.1016/j.scitotenv.2017.02.075 .

Zangi-Kotler M, Ben-Dov E, Tiehm A, Kushmaro A (2015) Microbial community structure and dynamics in a membrane bioreactor supplemented with the flame retardant dibromoneopentyl glycol. Environ Sci Pollut Res Int 22 17615–17624. doi:10.1007/s11356-015-4975-8

Zejie W, Gurumurthy Dummi M., Yicheng W., Feng Z., 2017 Progress of air-breathing cathode in microbial fuel cells, *Journal of Power Sources*, Vol 356, Pages 245-255,

Zhang L, Chen F, Zeng Z, Xu M, Sun F, Yang L, Bi X, Lin Y, Gao Y, Hao H, Yi W, Li M, Xie Y. 2021: Advances in Metagenomics and Its Application in Environmental Microorganisms. Front Microbiol. 17;12:766364. doi: 10.3389/fmicb.2021.766364. PMID: 34975791; PMCID: PMC8719654.

Zhang T, Gannon SM, Nevin KP, Franks AE, Lovley DR (2010) Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. *Environmental Microbiology* 12(4) 1011–1020. https://doi.org/10.1111/j.1462-2920.2009.02145.x

Zhang, C., Qi, J., Cao, Y., 2014. Synergistic effect of yeast-bacterial co-culture on bioremediation of oil-contaminated soil. Biorem. J. 18, 136–146.

Zhang, S., Merino, N., Okamoto, A., Gedalanga, P., 2018. Interkingdom microbial consortia mechanisms to guide biotechnological applications. Microb. Biotechnol. 11, 833–847.

Zhang, T., Tremblay, P.L., Chaurasia, A.K., Smith, J.A., Bain, T.S., Derek L.R., 2013. Anaerobic benzene oxidation via phenol in Geobacter metallireducens. *Applied and Environmental Microbiology*. 79, 7800–7806. **DOI:** 10.1128/AEM.03134-13

Zhou, W., Zhu, L., 2005. Solubilization of polycyclic aromatic hydrocarbons by anionic–nonionic mixed surfactant. Colloids and Surfaces A: Physicochem. Eng. Aspects 255, 145–152. Vogl, A., Bischof, F., Wichern, M., 2016. Increase life time and performance of Microbial Fuel Cells by limiting excess oxygen to the cathodes. Biochem. Eng. J. 106, 139–146. https://doi.org/10.1016/j.bej.2015.11.015

Chapter 9: Appendices

Appendix 1: Result of the physicochemical properties of the polluted environmental samples

Report for the characterisation of the petroleum hydrocarbon polluted groundwater

Analytical Report Number: 21-67129

Lab Sample Number				1828621
Sample Reference				Water
Depth (m)				5.00
Analytical Parameter (Water Analysis)	**Units**	**Limit of detection**	**Accreditation Status**	
General Inorganics				
Sulphate as SO4	mg/l	0.045	ISO 17025	18.5
Nitrate as N	mg/l	0.01	ISO 17025	0.76
Nitrate as NO3	mg/l	0.05	ISO 17025	3.35
Speciated PAHs				
Naphthalene	µg/l	0.01	ISO 17025	56.7
Acenaphthylene	µg/l	0.01	ISO 17025	< 0.01
Acenaphthene	µg/l	0.01	ISO 17025	< 0.01
Fluorene	µg/l	0.01	ISO 17025	19.8
Phenanthrene	µg/l	0.01	ISO 17025	36.9
Anthracene	µg/l	0.01	ISO 17025	< 0.01
Fluoranthene	µg/l	0.01	ISO 17025	< 0.01
Pyrene	µg/l	0.01	ISO 17025	< 0.01
Benzo(a)anthracene	µg/l	0.01	ISO 17025	< 0.01
Chrysene	µg/l	0.01	ISO 17025	< 0.01
Benzo(b)fluoranthene	µg/l	0.01	ISO 17025	< 0.01
Benzo(k)fluoranthene	µg/l	0.01	ISO 17025	< 0.01
Benzo(a)pyrene	µg/l	0.01	ISO 17025	< 0.01
Indeno(1,2,3-cd)pyrene	µg/l	0.01	ISO 17025	< 0.01
Dibenz(a,h)anthracene	µg/l	0.01	ISO 17025	< 0.01
Benzo(ghi)perylene	µg/l	0.01	ISO 17025	< 0.01
Total PAH				
Total EPA-16 PAHs	µg/l	0.16	ISO 17025	113

Lab Sample Number				1828621
Sample Reference				Water
Depth (m)				5.00
Analytical Parameter (Water Analysis)	**Units**	**Limit of detection**	**Accreditation Status**	
Heavy Metals / Metalloids				
Iron (dissolved)	mg/l	0.004	ISO 17025	0.010
Fe2+	mg/l	0.2	NONE	< 0.20
Fe3+	mg/l	0.2	NONE	< 0.20
Mn (II)	mg/l	0.02	NONE	0.03
Mn (IV)	mg/l	0.02	NONE	< 0.02
Arsenic (dissolved)	µg/l	0.15	ISO 17025	0.41
Monoaromatics & Oxygenates				
Benzene	µg/l	1	ISO 17025	9.0
Toluene	µg/l	1	ISO 17025	< 1.0
Ethylbenzene	µg/l	1	ISO 17025	< 1.0
p & m-xylene	µg/l	1	ISO 17025	35.5
o-xylene	µg/l	1	ISO 17025	27.4
MTBE (Methyl Tertiary Butyl Ether)	µg/l	1	ISO 17025	< 1.0
Petroleum Hydrocarbons				
TPH1 (C10 - C40)	µg/l	10	NONE	130000

U/S = Unsuitable Sample I/S = Insufficient Sample

Report for the physicochemical properties of the petroleum hydrocarbon polluted soil and sediment samples
Analytical Report Number: 2167129

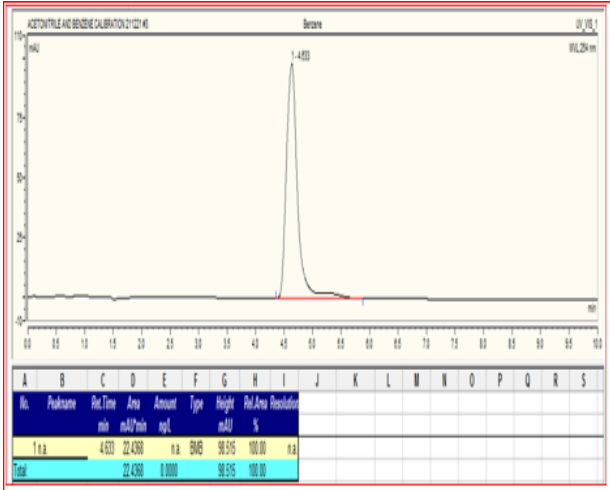
Lab Sample Number				1828619	1828620
Sample Reference				Polluted soil	Polluted sediment
Depth (m)				0.40	0.90
Analytical Parameter (Soil Analysis)	**Units**	**Limit of detection**	**Accreditation Status**		
Stone Content	%	0.1	NONE	< 0.1	< 0.1
Moisture Content	%	0.01	NONE	14	18
Total mass of sample received	kg	0.001	NONE	0.30	0.30
General Inorganics					
Total Sulphate as SO4	mg/kg	50	MCERTS	-	340
Total Sulphate as SO4	%	0.005	MCERTS	U/S*	0.034
Total Organic Carbon (TOC)	%	0.1	MCERTS	4.6	0.7
Water Soluble Nitrate (2:1) as NO3	mg/kg	2	NONE	5.3	2.5
Speciated PAHs					
Naphthalene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Acenaphthylene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Acenaphthene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Fluorene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Phenanthrene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Anthracene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Fluoranthene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Pyrene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Benzo(a)anthracene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Chrysene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Benzo(b)fluoranthene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Benzo(k)fluoranthene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Benzo(a)pyrene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Indeno(1,2,3-cd)pyrene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Dibenz(a,h)anthracene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Benzo(ghi)perylene	mg/kg	0.05	MCERTS	< 0.05	< 0.05
Total PAH					
Speciated Total EPA-16 PAHs	mg/kg	0.8	MCERTS	< 0.80	< 0.80
Lab Sample Number				1828619	1828620
Sample Reference				Polluted soil	Polluted sediment
Depth (m)				0.40	0.90

Analytical Parameter (Soil Analysis)	Units	Limit of detection	Accreditation Status		
	Heavy Metals / Metalloids				
Arsenic (aqua regia extractable)	mg/kg	1	MCERTS	U/S*	< 1.0
Iron (aqua regia extractable)	mg/kg	40	MCERTS	U/S*	2500
Fe2+	mg/kg	2	NONE	U/S*	< 2.00
Fe3+	mg/kg	2	NONE	U/S*	2490
Manganese (aqua regia extractable)	mg/kg	1	MCERTS	U/S*	14
Monoaromatic and oxygenates					
Benzene	µg/kg	1	MCERTS	< 1.0	< 1.0
Toluene	µg/kg	1	MCERTS	19	< 1.0
Ethylbenzene	µg/kg	1	MCERTS	< 1.0	< 1.0
p & m-xylene	µg/kg	1	MCERTS	45	< 1.0
o-xylene	µg/kg	1	MCERTS	53	< 1.0
MTBE (Methyl Tertiary Butyl Ether)	µg/kg	1	MCERTS	< 1.0	< 1.0
Petroleum hydrocarbon					
TPH C10 - C40	µg/kg	10	MCERTS	300000	< 10

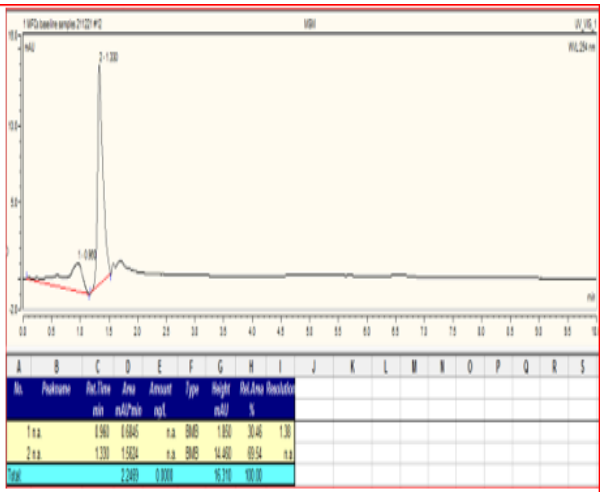
U/S = Unsuitable Sample
I/S = Insufficient Sample
* Analysis could not be completed due to sample matrix

Appendix 2: HPLC chromatogram results

HPLC resultss showing the retention time for benzene BES minimal defined medium components respectively.

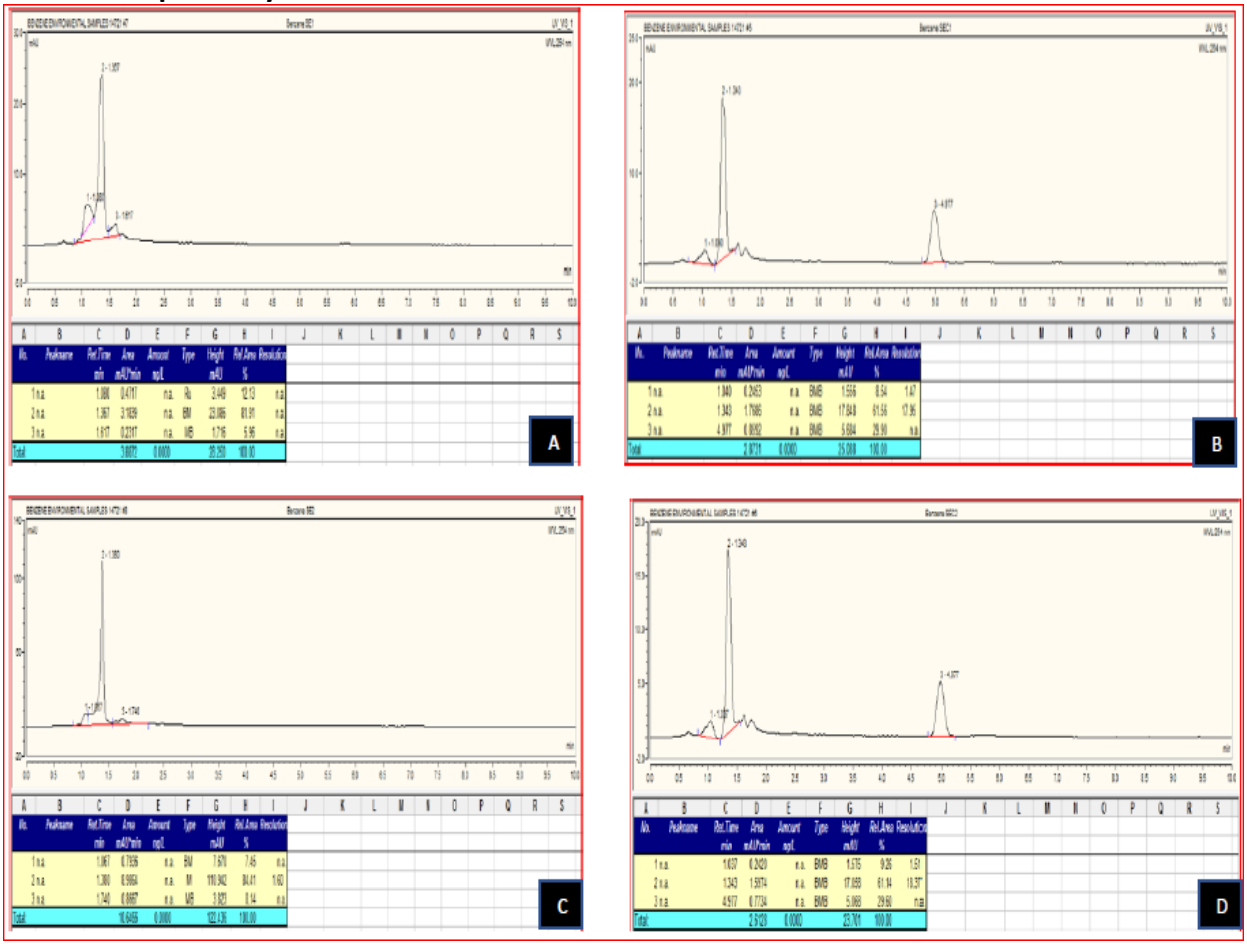


Highly concentrated benzene chromatogram to ascertain its retention time in minute

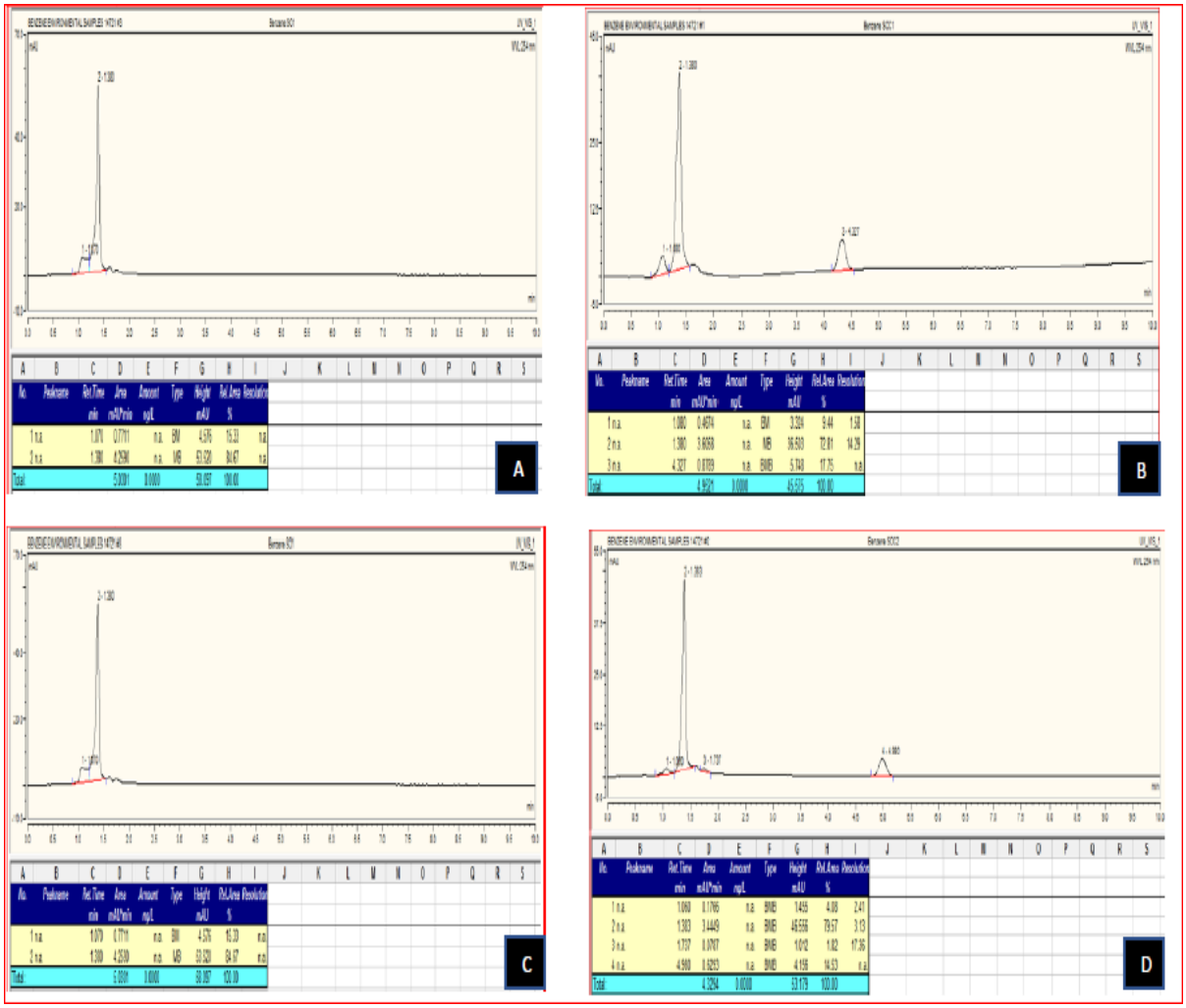


Chromatogram of BES minimal defined medium components to ascertain its retention time in minute

HPLC result picture labelled A and C show enrichment medium inoculated with polluted sediment samples while B and D are the negative control for iron III oxide and manganese IV oxide respectively



HPLC result picture labelled A and C show enrichment medium inoculated with polluted soil samples while B and D are the negative control for iron III oxide and manganese IV oxide respectively.


A
B
C
D

Appendix 3: NCBI blast results for the pure colonies isolated

NCBI blast results for the pure colonies isolated from the petroleum hydrocarbon polluted groundwater.

Isolate code	**Scientific name of highly similar organism**	***Max Score***	***Total Score***	***Query Cover***	***Per. Ident***	***Acc. Len***	***Accession***
5	*Pseudomonas sp. LCR71*	*2089*	*2089*	*80%*	*98.64%*	*1387*	*FJ976580.1*
A	*Pseudomonas azotoformans*	*2041*	*2041*	*83%*	*97.10%*	*1228*	*OP263598.1*
1	*Clostridium senegalense JC122*	*2069*	*2069*	*80%*	*99.39%*	*1462*	*NR_125591.1*
2K	*Pseudomonas sp.*	*2008*	*2008*	*89%*	*98.51%*	*1197*	*MG833390.1*
2N	*Pseudomonas libanensis*	*2015*	*2015*	*96%*	*97.79%*	*1332*	*OP104209.1*
2O	*Pseudomonas sp.*	*1995*	*1995*	*87%*	*97.37%*	*1495*	*MN841966.1*
2Q	*Pseudomonas sp. SCB32*	*2185*	*13103*	*90%*	*98.55%*	*6311241*	*CP045118.1*
2W	*Pseudomonas sp. J380*	*2025*	*12144*	*91%*	*98.52%*	*6261650*	*CP043060.1*
2J	*Pseudomonas sp. SCB32*	*2006*	*12028*	*96%*	*98.51%*	*6311241*	*CP045118.1*

NCBI blast results for the sequence of pure colonies isolated from the petroleum hydrocarbon polluted soil.

Isolate code	Scientific name of highly similar organism	Max Score	Total Score	Query Cover	Per. Ident	Acc. Len	Accession
2B	*Pseudomonas sp. CSJ-3*	2097	2097	96%	96.97%	1435	KF861966.1
B	*Clostridium beijerinckii*	2098	2098	83%	98.10%	1424	KF892545.1
J	*Ewingella americana*	2013	2013	80%	98.02%	1499	MN826581.1
F	*Pseudomonas paralactis*	2050	2050	78%	98.54%	1401	MW207986.1
9	*Ewingella americana*	2030	2030	80%	98.44%	1499	MN826581.1
E	*Pseudomonas fluorescens*	1995	1995	75%	98.24%	1412	MN173420.1
8	*Enterobacter ludwigii*	1995	1995	77%	98.84%	1492	MT180566.1
10	*Ewingella americana*	2026	2026	81%	98.36%	1499	MN826581.1
M	*Pseudomonas azotoformans*	2089	2089	85%	97.94%	1337	MN877361.1
C	*Pseudomonas azotoformans*	2039	2039	78%	98.36%	1228	OP263598.1
1E	*Ewingella americana*	1989	1989	88%	98.84%	1499	MN826581.1
1F	*Microvirgula aerodenitrificans*	1722	1722	96%	97.35%	1460	MT367755.1
2M	*Microvirgula aerodenitrificans*	1929	1929	96%	96.21%	1460	MT367755.1
2S	*Microvirgula aerodenitrificans*	1978	1978	89%	97.91%	1460	MT367755.1
1G	*Rahnella sp. UIWRF1115*	1954	1954	88%	97.54%	1372	KR189413.1
1J	*uncultured bacterium*	1930	1930	79%	98.98%	1465	KT029431.1
1D	*Ewingella americana*	1965	1965	91%	98.14%	1499	MN826581.1
2P	*Pseudomonas sp.*	1901	1901	84%	98.43%	1197	MG833390.1
1I	*Clostridium sp. Cd6*	1962	1962	90%	98.39%	1494	AB673452.1

NCBI blast results for the sequence of pure colonies isolated from the petroleum hydrocarbon polluted sediment.

isolate code	Scientific name of highly similar organism	Max Score	Total Score	Query Cover	Per. Ident	Acc. Len	Accession
2A	*Pseudomonas azotoformans*	1969	1969	98%	95.98%	1337	MN877361.1
2D	*Clostridium butyricum*	1797	1797	87%	96.12%	1410	MT510294.1
1B	*Clostridium sp. Marseille-P8840*	1831	1831	85%	96.99%	1527	LR738916.1
2U	*Lacrimispora saccharolytica*	1796	10715	84%	96.12%	4678070	CP070235.1
G	*Clostridium butyricum*	1993	1993	82%	97.76%	1410	MT510294.1
2E	*Clostridium butyricum*	1842	21997	81%	97.25%	3782283	CP073277.1
1R	*Pseudomonas sp.*	1160	1160	66%	94.14%	1403	KX066820.1
1Q	*Pseudomonas sp. sw6*	1537	1537	90%	94.06%	1317	EF027000.1
1L	*Clostridium butyricum*	854	854	65%	84.24%	1410	MT510294.1
1O	*Pseudomonas sp.*	1179	1179	80%	93.63%	1458	MW091474.1
2X	*Alphaproteobacteria bacterium*	1690	1690	96%	97.01%	1273	ON329562.1
2F	*Pseudomonas chengduensis*	1934	1934	90%	98.12%	1239	MN877372.1
1M	*Lacrimispora celerecrescens*	1958	1958	87%	98.31%	1433	MT264995.1
2V	*Nanobacterium sp. Persian TH hmb*	2084	2084	93%	97.31%	1290	KX156617.1
2T	*Pseudomonas sp.*	1753	1753	88%	99.18%	1235	MH236001.1
1N	*Pseudomonas alcaliphila*	1934	1934	90%	97.87%	1403	MH127731.1