



**Bioelectrochemical remediation of oil-contaminated marine sediment via novel microbial electrochemical snorkel**

Lappeenranta – Lahti University of Technology LUT

Master's Degree Program in Chemical and Process Engineering, Master's thesis

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Examiners: Professor Eveliina Repo

Professor Amit Bhatnagar

## ABSTRACT

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Following an unintentional oil spill, marine sediments serve as an ultimate sink for a variety of human-made organic pollutants, especially petroleum hydrocarbons. The activity of indigenous microfloral populations connected to biogeochemical cycles, in which sulfur is transported to and from its oxidized and reduced species, is crucial to the degradation of these compounds. This study presents an additively manufactured electrode made of super nickel alloy Inconel 718 ("the electrochemical snorkel") inserted into oil- contaminated sediment originating from the Baltic Sea in the north of Finland, consequently establishing an electrochemical contact between the anaerobic polluted marine sediment and the aerobic superjacent water, has a significant influence on the microbial activity in the core of the sediment. The electrochemical snorkel enhanced organic pollutant oxidation-driven sulfate reduction activities. Two types of chemical analyses have been implemented to investigate this phenomenon, proposing that the snorkel enhanced the reduction of the sulfate anions in all samples and that the electrode surface patterning has given better hydrocarbons degradation potential to the electrode possessing a microstructure. Overall, the findings of this study can be considered as a step along the way of providing industrially scalable materials to manufacture the microbial electrochemical snorkel which would positively impact benignly counteracting oil spills in water bodies.

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## ABBREVIATIONS

AM	Additive Manufacturing
BES	Bioelectrochemical Systems
BOD	Biological Oxygen Demand
BTEX	Benzene, Toluene, Ethylene, and Xylene
CEM	Cation Exchange Membrane
COD	Chemical Oxygen Demand
EAB	Electroactive Bacteria
FS	Flat Snorkel
GC-FID	Gas Chromatography with Flame Ionization Detector
IC	Ion Chromatography
MFC	Microbial Fuel Cell
MEC	Microbial Electrochemical Snorkel
MES	Microbial Electrochemical Snorkel
MS	Microstructured Snorkel
OER	Oxygen Evolution Reaction
PAH	Polycyclic Aromatic Hydrocarbons
PEM	Proton Exchange Membrane
PHs	Petroleum Hydrocarbons
PCBs	Polycyclic Biphenyls
ROI	Radius of Influence
SSA	Specific Surface Area
SRB	Sulfate Reducing Bacteria
TEA	Terminal Electron Acceptor

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## INTRODUCTION

Crude oil normally consists of thousands of chemicals mostly containing aliphatic and aromatic hydrocarbons, and when they contaminate the environment, these chemicals account for a variety of harmful ecological implications (Daghio, et al., 2017). Oil spill refers to the inadvertent or intentional discharge of oil into the environment, and when exceeding 100 thousand gallons it is termed as "Major" (Dhaka & Chattopadhyay, 2021). After an oil spill, the sediment accumulates several hydrophobic organic pollutants, and as a result of the prevalence of anoxic conditions that severely hamper the oxidative biodegradation of these contaminants, it transforms into a major sink and source of contamination, consequently, posing threats to the human and surrounding environment especially when atmospheric conditions redistribute the contamination into the water column (Lofrano, et al., 2016; Bellagamba, et al., 2017). Besides the marine ecosystem, marine oil spillages and their subsequent harmful impacts have a deleterious effect on linked activities including fishing, marine agriculture, tourism, and the petroleum sector (Dhaka & Chattopadhyay, 2021). Furthermore, oil spills result in significant economic burdens and judicial disputes for the authorities liable and corporations. A notorious example was the Deepwater Horizon oil spill which was one of the worst ecological catastrophes in human history that led to the release of more than 4.9 million barrels of crude petroleum in the Gulf of Mexico (Chen, et al., 2019). Hence, controlling spillages is a primary goal., especially in vulnerable zones where spills can cause catastrophic or irreversible consequences, e.g., the Arctic (Eide, et al., 2007). Nevertheless, unintentional oil spillages could still take place and therefore an immediate action must be taken by using proper remediation countermeasures. The methods used to tackle marine oil spills are physicochemical and biological (bioremediation) and they could be used separately or in tandem (Alvarez & Illman, 2006). In situ remediation techniques are more favored than ex-situ methods as they are associated with less sediment mobilization (Lofrano, et al., 2016). On the other hand, compared to physicochemical techniques, bioremediation methods are more innocuous and relatively cost-effective, making them a sustainable solution for the remedy of polluted sediments (April, et al., 2000). Among bioremediation approaches, bioelectrochemical systems (BES) have been attracting increasing interest for polluted sites remediation as they utilize the microflora's ability to



make a direct electrochemical connection with solid electrodes that are immersed in a polluted area, to generate electricity via redox reaction (Li & Yu, 2015; Bellagamba, et al., 2017) and they were initially implemented in wastewater treatment (Logan, et al., 2006). Normally, a bioelectrochemical system consists of two electrodes (e.g., anode and cathode) connected through a wire and separated by an ion conductive medium, however; in 2008, a very simple model has emerged in which the anode and cathode are coupled and mostly made of the same material (Bergel, et al., 2008). From a design point of view, it was considered as a short-circuited microbial fuel cell and it was given the name 'Microbial Electrochemical Snorkel' (Santoro, et al., 2017). Despite no electricity generation is associated with this technology as a result of the anode and cathode being coupled, it is claimed to sustain the highest possible electrochemical reaction rate (Hoareau, et al., 2019), which makes it extremely beneficial when the goal is to remediate the sediment after oil spillages (Wu, et al., 2018).

The main objective of this work is to utilize the aforementioned concept of the microbial electrochemical snorkel (MES) in developing a novel electrode that can ameliorate the anaerobic biodegradation of oil-contaminated marine sediments. This is done through reviewing the previous work on the same topic to develop an electrode that is chemically resistant, biocompatible with the indigenous microbial community in benthic sediments, and has the potential to be scaled up. In previous work, a snorkel made of graphite was used for the same purpose and showed high potential for TPH biodegradation in marine sediment, however; graphite is considered as one of the softest materials (Kharisov & Kharissova, 2019) which makes it unfavorable for real-life application. Half the number of the developed electrodes have surface patterning in the form of micropillars, and that is to investigate the improvement potential in the efficiency of TPH biodegradation in the contaminated sediment compared to the non-patterned (e.g., flat) electrode. A set of sacrificial microcosms are prepared for the artificially oil-spiked marine sediment together with the novel snorkel and seawater and incubated for 104 days. GC-FID and IC were used to analyze TPH in the sediment and sulfate ions in seawater respectively. X-ray photoelectron spectroscopy (XPS) was supposed to be employed to analyze the surface chemistry of the snorkel's surface, however; because of time constraints, it will be postponed to further research work.

# 1 LITERATURE REVIEW

The literature review first starts with a short review of PHs, how they represent an ecological threat, and their fate. A brief review of the existing countermeasures for treating PHs highlighting the emergence of BES as an efficient technique in PHs elimination. A summary for the previous research related to BES in the remediation of oil-contaminated marine sediment was given, highlighting the use of microflora in the process, and the characteristics of BES, their advantages, and drawbacks. The MES is described and how it was implemented in the previous literature work. Finally, the rule of AM technology in this work is presented and the selected material for the electrode manufacture.

## 1.1 Petroleum hydrocarbons description and classification

Petroleum compounds consisting almost entirely of carbon and hydrogen are known as Petroleum Hydrocarbons (PHs). They represent a broad spectrum of products, such as gasoline, diesel fuel, etc. which have a different molecular weight of individual hydrocarbons.

The PHs can be subdivided into the following categories: (Fig.1)

- a) Alkanes (or paraffins): Saturated hydrocarbons, consisting of only carbon and via single bonds. In these compounds, the carbon atoms are connected via unbranched or branched chains, hence they are called aliphatic. Butane, Hexane, Heptane, Octane, and Decane are examples of this category (Kuppusamy, et al., 2020).
- b) Cycloalkanes (or naphthalenes): Saturated hydrocarbons that have a ring-type structure. Examples are Cyclobutane, Cyclopentane, Cyclohexane, and Cycloheptane (Kuppusamy, et al., 2020).
- c) Alkenes (or olefins): Unnsaturated compounds with a minimum of one carbon-to-carbon double bond. Examples are Hexene, Pentene, and Ethylene (Kuppusamy, et al., 2020).
- d) Aromatic Hydrocarbons (Arenes): Unsaturated hydrocarbons containing at minimum one benzene ring in their structure. Common examples are Benzene, Toluene, Ethylene, and Xylene (BTEX). This group involves compounds that contain not less than three aromatic rings, these are known as polycyclic aromatic

hydrocarbons (PAH). Examples are Naphthalene and Anthracene (Kuppusamy, et al., 2020).

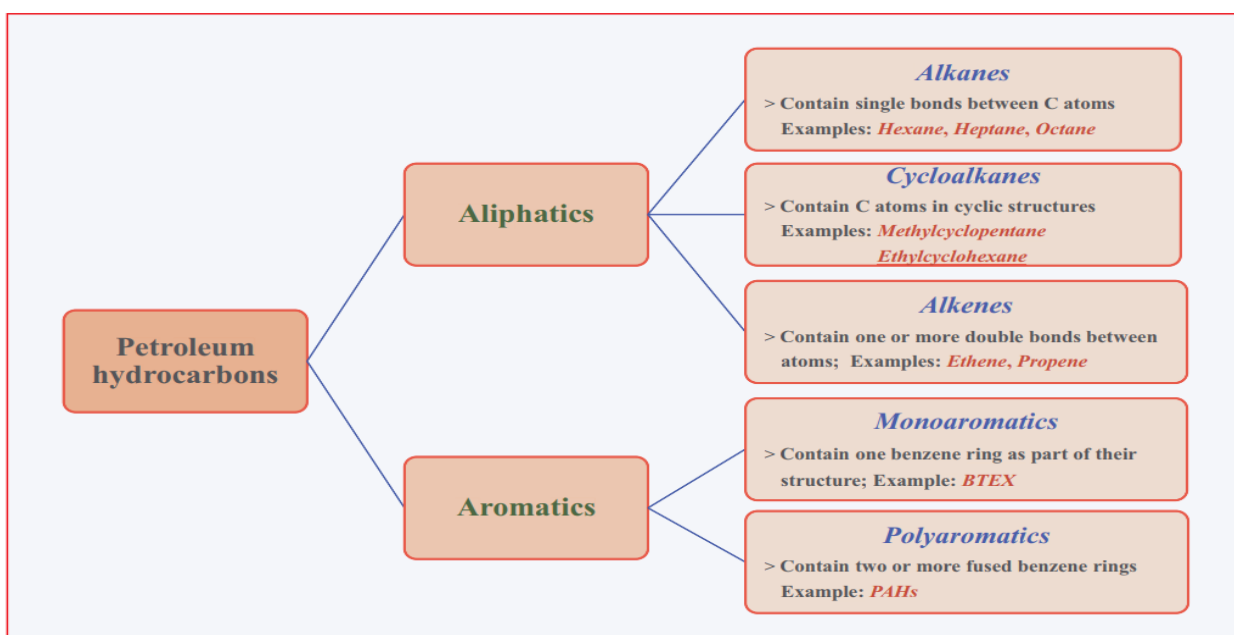


Figure 1. Classification of PHs (Kuppusamy, et al., 2020)

## 1.2 PHs existence in nature as a contamination threat:

PHs pose a threat to the environment when they exist in sediment, soil, water, and air. The degree of toxicity of petroleum hydrocarbons varies based on their chemical structure, characteristics of the compound fractions, the route, level, and time of exposure (Ossai, et al., 2020).

Since they are components of crude oil and its derivative materials, PHs can be found in several locations, such as refineries, industrial locations where they are used as feedstock, and locations using hydrocarbons as fuel or lubricants and filling stations (Kuppusamy, et al., 2020). They also might exist due to the oil spill arising from marine shipping. The presence of PHs in the sediment and marine ecosystem can originate from marine transportation, river runoff, adsorption, precipitation, natural seeps, municipalities, commercial wastes, and overseas manufacturing (Kuppusamy, et al., 2020; Zhou, et al., 2014; Yang & Xu, 2019)

### 1.3 The fate of PHs in aquatic systems

When liquid PHs are discharged into the surrounding environment as a consequence of industrialization and other human-made activities, they cause contamination of severe environmental threats. Once the oil spills, PHs are commonly distributed and flow in and on the surfaces of polluted areas and subject to numerous Physico-chemical variations. A collective term is used to describe these processes, i.e., “weathering” and it determines the “fate of the oil” (Mishra & Kumar, 2015). Figure 2. Many factors determine the rate and significance of the technologies, for instance, the amount of spill, the oil's original physical and chemical specifications, the current ecological circumstances, and the possibility of the oil staying in or slipping away from the spilled site. (Kuppusamy, et al., 2020).

### 1.4 The threat emanating from marine sediments

Studies showed that the sediment constitutes the most problematic component in aquatic environmental remediation since a large number of water, earth, and atmospheric pollutants ultimately accumulate through different atmospheric or geochemical processes in aquatic sediments (e.g., precipitation, surface runoff, and adsorption) (Li & Yu, 2015). The sediment is also the source of most recalcitrant pollutants, including polycyclic aromatics (PAH), polycyclic biphenyls (PCBs), and heavy metals (Sprovieri, et al., 2006). Sediment accumulates recalcitrant contaminants which in the long term are continuously released into the water body after being deposited in sediment. As a result, sediment serves as both a sink and a contamination zone in the marine environment. (Yan, et al., 2017)

### 1.5 Existing countermeasures for treating petroleum hydrocarbons

There are two main categories of countermeasures used in the abatement of PHs, Physicochemical and Biological methods. In the text mentioned below both are introduced.

#### 1.5.1 Physicochemical treatment processes:

One of the deep-rooted physicochemical techniques for the detoxification of petroleum hydrocarbons from marine locations is via confining the oil spill using booms and skimmers. A boom is a mechanical barricade that floats on the water surface, built to stop or deflect the movement of oil on water. Booms look like a vertical curtain with segments up and beneath the water surface, Figure 3. Skimmers are mechanical instruments developed for removing oil from the water surface. They largely differ in terms of size, application, and capacity, and recovery performance (Fingas, 2011). This decontamination method is practical and rapid; however, it is costly and mostly utilized as an emergency measure to oil spillages in marine

environments (Dhaka & Chattopadhyay, 2021). The main downside of this method is that it only eliminates oil spills from the polluted area without any sort of treatment, which will be done afterward via other treatment methods. Besides, it is limited in use on surface water (Adelaja, 2015).

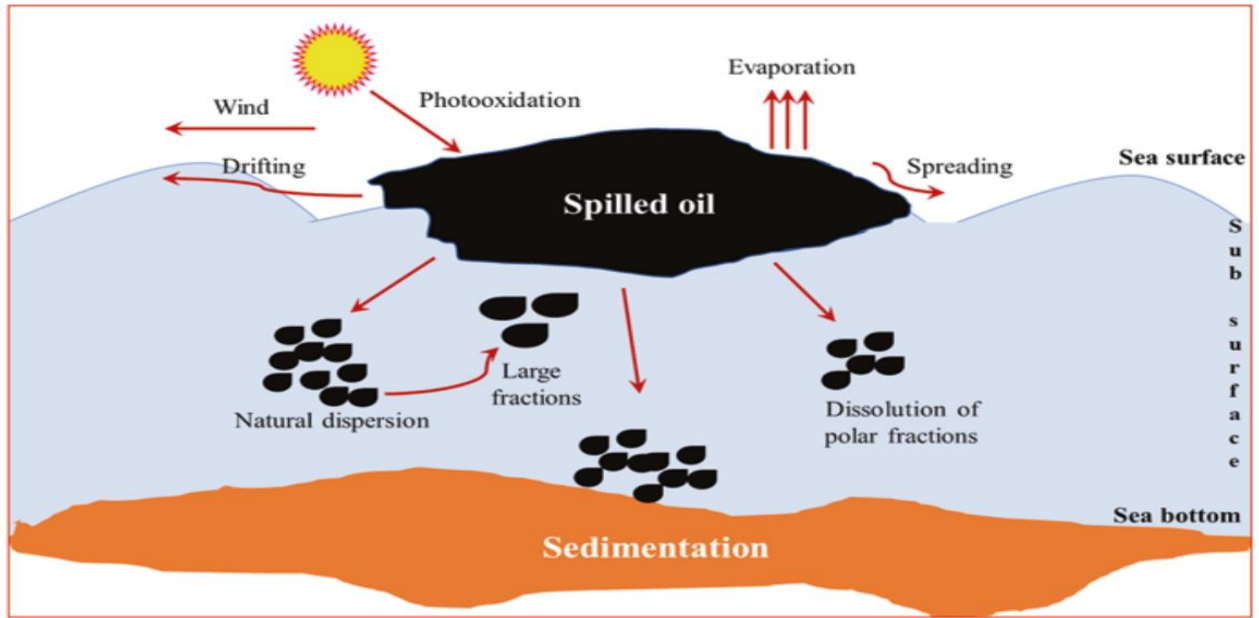


Figure 2. The weathering phenomenon of oil spill in the sea (Xie, et al., 2007; Kuppusamy, et al., 2020)

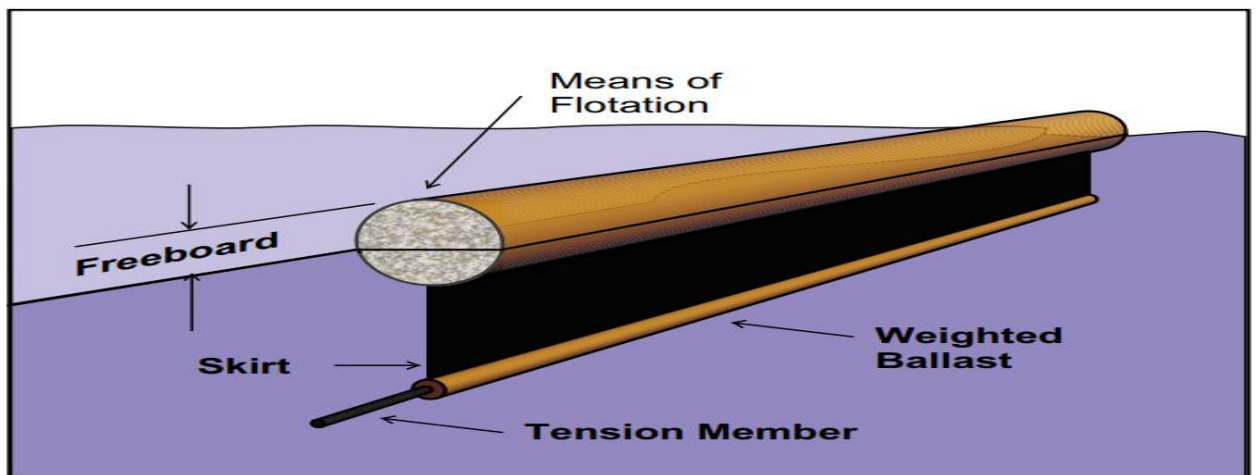


Figure 3. Simple boom structure (Fingas, 2011)

Another physicochemical process is the adsorption of PHs, which utilizes an array of adsorbents, including activated carbons, zeolites, and copolymers (Ahmadun, et al., 2009). Nevertheless, one of the disadvantages of this process is the spent adsorbents that need to be disposed of. Another drawback is the functional specificity of some of these adsorbents, such as zeolites and copolymers. Furthermore, the high price of these adsorbents and the difficulty and unsustainability in regenerating them make them less favored (Frick, et al., 1999).

One of the in-situ chemical methods is the use of oil dispersants that have been utilized for decades for alleviating the implications of the marine oil spill (Dave & Ghaly, 2011; Kleindienst, et al., 2015). This works by minimizing the size of oil droplets, altering the physicochemical properties of the oil surface, and increasing the distribution of oil in the water column, to make the oil spill less visible (Gong, et al., 2014). However, dispersants and oil that are dispersed in the ocean are toxic to marine life (Mu, et al., 2014)

Another form of chemical remediation that has been applied is chemical oxidation, which implements chemical oxidants in converting sediment pollutants to less detrimental chemical compounds. This is done by introducing chemical oxidants into the subsurface (Lofrano, et al., 2016). The process involves the use of chemical oxidants such as hydrogen peroxide, Na-permanganate, and Na-persulfate (Shih, et al., 2016). Nevertheless, the resulting oxidation reactions are rapid and short-acting, several doses of the chemical should be added, and the formation of unfavorable by-products may be inevitable, which can hamper further degradation. Although these oxidants are powerful in the mineralization of numerous harmful toxic compounds, the need to add chemicals to the sediment and their repercussions on the environment render the use of this technique less favored (Lofrano, et al., 2016).

Dig and dump, soil cleaning, and soil vapor extraction are typical physicochemical treatment methods for remediating PHs-contaminated sites, while thermal treatment processes favored mainly by regulators comprise thermal desorption and incineration. These technologies, however, are costly, non-invasive, and can lead to serious environmental consequences and incomplete degradation of PHs (Kuppusamy, et al., 2020).

### 1.5.2 Biological treatment processes

Owing to the aforementioned challenges encountered when using physicochemical remediation strategies, biological methods were sought as an alternative strategy, e.g., bioremediation. By using pollutants as an energy and carbon source, bioremediation takes advantage of the broad metabolic diversity of microflora for the abatement of organic pollutants (Daghio, et al., 2017). Compared to physicochemical methods, It is typically affordable and allows for the ultimate degradation of contaminants (Chandrasekhar, et al., 2020). Nevertheless, it takes a longer time for ultimate remediation to be achieved (Atlas, 1995). Bioremediation aims to promote the removal of pollutants by overcoming microbial metabolism limitations that would alternatively derail the abatement of contaminants. A standard approach is to provide electron donors to promote halogenated compound

mineralization (i.e., reduction) or electron acceptors to induce non-halogenated compound degradation (i.e., oxidation) (Alvarez & Illman, 2006).

Due to being environmentally benign, relatively affordable, and using equipment that needs less maintenance., bioremediation, i.e., the utilization of microflora to detoxicate or eliminate contaminants, represent a promising technique (Kuppusamy, et al., 2020). Additionally, Bioremediation is one of the approaches to mitigating the issue of PH pollution with green technologies (Zaida & Piakong, 2019).

Bioremediation techniques can be classified into two categories, natural attenuation, which involves solely monitoring the occurrence of natural remediation without any other human intervention, and engineered bioremediation such as bioaugmentation (the addition of allochthonous active microorganisms for contaminant mineralization), biostimulation (autochthonous microbial consortia that remove contaminants are stimulated to eliminate the factors that limit their growth), biopiling (polluted soil is piled up, and aerobic microbial activity within the soil is promoted by providing oxygen, minerals, nutrients, and moisture), bioventing (by introducing oxygen, via vacuum pumps, to allow microorganisms to biodegrade organic pollutants), and phytoremediation (to abate and/or eliminate pollutants from sediment by using macrophytes and/or algae). All these are among the most commonly used bioremediation methods in PH remediation (Kuppusamy, et al., 2020). The main downside of the natural attenuation method is that achieving good biodegradation efficiency might take years (Mulligan & Yong, 2004), whereas the engineered bioremediation methods can minimize the treatment duration from years to days based on the nature of the polluted site (Talley & Sleeper, 2006). These engineered bioremediation strategies fall into two categories, ex-situ and in-situ, but regardless of their merits, they are costly and are commonly associated with various types of environmental hazards ( Adelaja, 2015).

Aerobic bioremediation is a more favorable option than its anaerobic counterpart, as it accelerates the rate of hydrocarbons' degradation. Hence, A modular slurry system is an example of several engineered solutions that have been introduced to supplement the polluted sediments with oxygen. This system has been developed by Genovese and her research group to provide in situ polluted sediments' aeration while reducing the hazard of transmitting the pollution beyond the treatment area (Genovese, et al., 2014). Notwithstanding the great efficiency of the system in promoting the metabolism of aerobic hydrocarbon degraders and sediment detoxification, it had some drawbacks, such as being

labor-intensive and energy-demanding (Genovese, et al., 2014). Other strategies involve supplying the contaminated sediment with compounds that can liberate oxygen (e.g., chemicals containing calcium peroxide) (Abdallah, et al., 2009). However, the efficiency of this strategy is negatively affected by the expeditious oxygen uptake through reactions with reduced ions (e.g.,  $\text{Fe}^{2+}$ ,  $\text{S}^{2-}$ ), as well as the non-easy control of oxygen liberation over time (Borden, et al., 1997).

Microbial metabolism is the main driver for contaminant mineralization in sediments. The interaction between the bioavailability of terminal electron acceptors (TEA) (e.g., oxygen, nitrate, sulfate) and hydrocarbons, as electron donors and sources of carbon for the microorganisms, is a major aspect of how sediment bioremediation techniques would perform (Lu, et al., 2014).

## 1.6 The role bioelectrochemical systems (BES) play in the abatement of PHs

Due to the limitations associated with conventional petroleum hydrocarbon treatment methods, there is a need for more affordable, reliable, and environmentally benign technologies for the detoxification of polluted PH sites considering the shortcomings related to conventional processes of petroleum hydrocarbons treatment. Bioelectrochemical systems have been proposed as a feasible means to oxidize different organic wastes accompanied by electricity generation (Rozendal, et al., 2008)

### 1.6.1 BES types and historical overview

Bioelectrochemical systems are distinct and evolving technologies used to catalyze redox reactions in electrochemical cells using microbes attached to one or both electrodes (Rabaey, et al., 2009). The standard configuration of the reactor comprises an anodic and a cathodic compartment, isolated by an ion conductive medium (Daghio, et al., 2017).

They are classified into two categories, Microbial Fuel Cells (MFCs), and Microbial Electrolysis Cell (MECs). If the entire reaction is thermodynamically spontaneous and generates electricity, a BES is referred to as an MFC. In contrast, when electricity is provided to initiate a non-spontaneous electrochemical reaction, it is known to be a MEC (Adelaja, 2015).

MFCs are able to harness microflora, e.g., *Geobacter* spp, *Shewanella* spp, *Pseudomonas* spp, and *Rhodospirillum rubrum* in axenic (a single or a variety of strains of the same culture) or mixed cultures. They stimulate the anodic oxidation of substrates to concomitant biogenic



electricity production when connected to a load/resistor through an external cathode circuit. Protons are converted to vapor near the cathode. The electricity produced in BESs is an indicator of the organic substrate's transformation/biodegradability at the anode (Raghavan, et al., 2017). MFC techniques are modern and advanced modalities used extensively in the treatment of wastewater from various sources with the production of renewable power. (Patil, et al., 2009). Apart from electricity generation, MFCs can be utilized in agricultural wastewater treatment, toxic gas treatment, breakdown of petroleum compounds, and methane and hydrogen production (Yi, et al., 2009; Evelyn, et al., 2014), industrial and dairy wastewater treatment (Mohan, et al., 2010; Du, et al., 2007) and leachate treatment (Choi & Ahn, 2015).

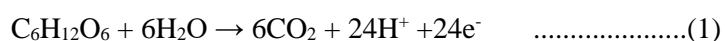
The first work on MFCs dates back to 1911 by M.C. Potter, a Botany Professor at Durham University, who investigated the breakdown of organic matter by microbes. He proved that organisms could deliver current and generate voltage. (Potter, 1908). More attention was paid to this field by Barnet Cohen as he made several microbial half fuel cells when linked in series produced over 35 volts, with a low amount of current of 2 milliamperes (Cohen, 1931)

In 1911, the Byung Hong Kim group (Korean Institute of Science and Technology, South Korea) developed a mediator-free MFC. They found, using electrochemical techniques, that *Shewanella* sp. can initiate electrochemical reactions. This result was a breakthrough in MFC, as it improved the commercial viability by removing expensive chemical mediators (Byung-Hong, et al., 1999).

### 1.6.2 Working principle of MFCs

MFCs are electrochemical devices mediated by microorganisms, which oxidize organic substrates through catabolism to generate electricity in a highly efficient manner for long time spans. In the presence of acetate as a substrate, the reactions taking place in anodic and cathodic compartments are as follows ( Adelaja, 2015):

#### **Anodic chamber:**



#### **Cathodic chamber:**



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

A typical MFC is made up of single anodic and cathodic compartments split by a proton/cation exchange membrane. MFC's basic steps are as follows (Sivasankar, et al., 2018): Initially, the organic substrate converts into protons and electrons in the cathode compartment. In the anode compartment, the microorganisms initiate the decay of biomass/substrate, delivering hydrogen ions and electrons. The electrons pass either directly or by mobile electron shuttles (mediators) to the electrodes of the bacterial cell. Through the external circuit, these electrons are ferried from the anode to the cathode. Instantaneously, protons move from the anode to the cathode. The PEM/CEM facilitates proton migration, while the electrons migrate through electrodes from the anode to the cathode chamber. An electrical current is generated while flowing through the electrical circuit. As hydrogen ions migrate from the anode to the cathode, a high electrochemical gradient is created near the anode. In the cathode chamber, the oxygen reduction into water, accepting electrons and protons facilitate further diffusion of hydrogen ions from anode to cathode (Logan, et al., 2005; Du, et al., 2007; Kumar, et al., 2016). Figure. 4 shows the overall process and the different types of anode and cathode chambers.

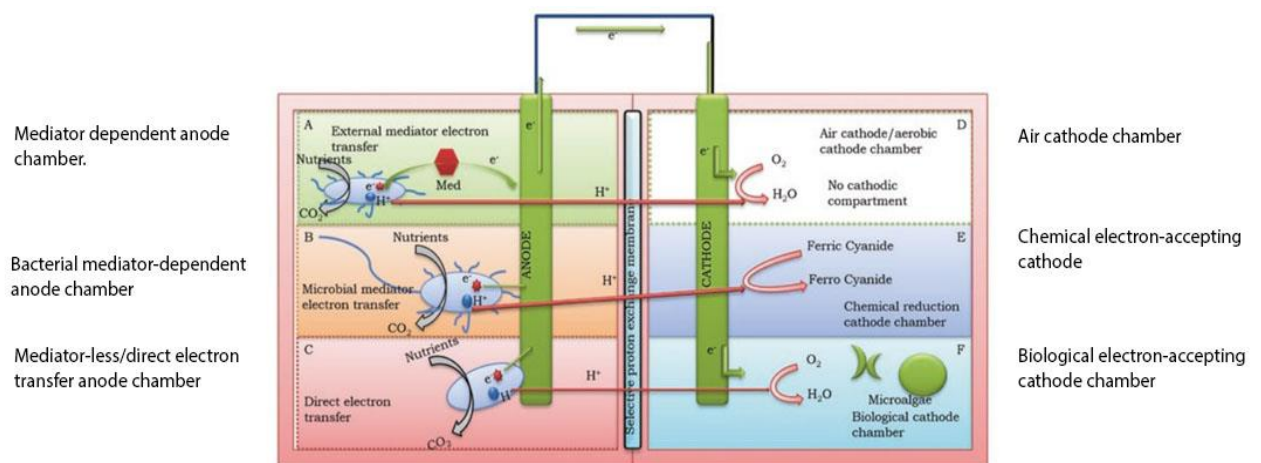


Figure 4. Different microbial fuel cell types (Sivasankar, et al., 2018)

### 1.6.3 How electrons transfer to solid electrodes

Electrons can be ferried between an electrode and electroactive bacteria (EAB) within the EAB biofilms either directly or indirectly (Kondaveeti, et al., 2018). These electroactive bacteria can be also called exoelectrogens (Logan, 2010), electricigens (Lovley, 2006), or anode-respiring bacteria (Torres, et al., 2010). Several types of bacteria may create their own

redox mediators, such as *Pseudomonas aeruginosa*, *Shewanella oneidensis*, and *Pseudomonas alcaliphila* (Santoro, et al., 2017). Electron transfer can be done via two paths (Fig. 5); (i) indirectly through oxidizing by-product of bacterial metabolism. An example of this is when fermentative bacteria form hydrogen which is then oxidized on the anode surface (Niessen, et al., 2006), (ii) directly via direct contact between the outer membrane of the bacteria and the surface of the anode (Bond, et al., 2002). Another form of direct transfer was described in 2005, which occurs through extra cellular conductive appendages called conductive pili or bacterial nanowires (Reguera, et al., 2005).

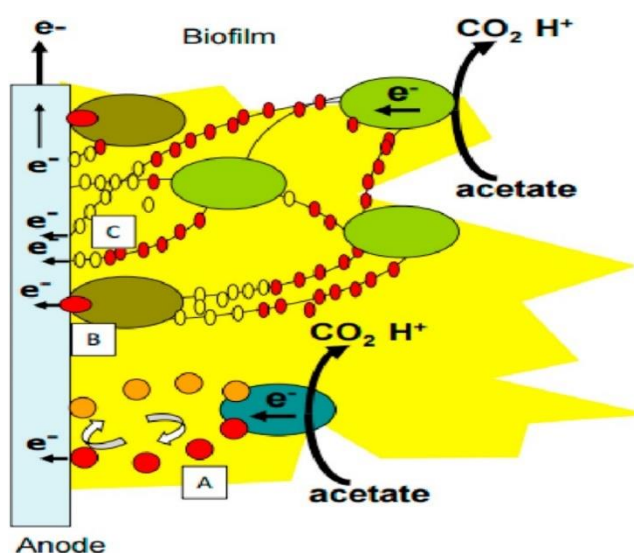


Figure 5. Electron transfer mechanisms:(a) Indirect transfer by mediator or fermentation products;(b) direct transfer by cytochrome protein;(c) direct transfer by conductive pili. (Santoro, et al., 2017)

Biocatalysts used in MFC anodes can be divided into two types, electrogenic and non-electrogenic microorganisms (Sacco, et al., 2017). Electrogenic bacteria are able to reduce external solid electron acceptors. In the anaerobic environment of microbial fuel cells (MFCs), they can oxidize compounds effectively while generating an electrical current (Kiseleva, et al., 2015). So far, different species of electrogenic bacteria have been reported, these include species of *Geobacter* (Bonanni, et al., 2013), species of *Shewanella*, species of *Rhodospirillum rubrum* (Chaudhuri & Lovley, 2003), species of *Escherichia coli*, species of *Pseudomonas*, most species of Proteobacteria and Firmicutes phyla (Rabaey, et al., 2004; Park & Zeikus, 2003), *Aeromonas hydrophila*, *Clostridium butyricum* and *Enterococcus gallinarum* (Rinaldi, et al., 2008). Non-electrogenic bacteria can break down complex organic molecules into more basic metabolites that electroactive bacteria can easily digest in a syntrophic process without electricity generation, e.g., fermentative bacteria and methanogens (Adelaja, 2015).

#### 1.6.4 Anodic oxidation and oxygen generation in BES

Lately, multiple researches have utilized BES systems to promote anaerobic crude oil oxidation as well as crude oil-derived compounds. Some of these studies are listed in Table.1 which were performed in marine sediment samples. The BES' anode is often employed to attract electrons released through organic pollutant oxidation (Daghio, et al., 2017). It can be embedded in anaerobic underwater sediment and has an electrical connection with a cathode positioned in the oxygenated water (Daghio, et al., 2017). The anode can be preinoculated or the resident microorganisms possessing electron transfer capabilities can colonize its surface. The anaerobic oxidation of the pollutants releases electrons which move to the cathode by means of electrical connection in the oxic water column where they could be utilized for oxygen reduction (Lovley & Nevin, 2011). Benzene was mineralized in BES anode by diversified bacterial populations in polluted sediment (Zhang, et al., 2014). Total petroleum hydrocarbon (TPH) degradation in marine sediments has been thoroughly reviewed in several studies (Viggi, et al., 2015; Li & Yu, 2015; Morris & Jin, 2012). The initiation of the degradation pathway is a significant barrier to electrochemical anaerobic hydrocarbons' bioremediation (Bertrand, et al., 2015). Under aerobic conditions, degradation starts with oxygenase enzyme stimulating the addition of hydroxyl groups (Daghio, et al., 2017). Less efficient processes usually occur in the prevalence of anaerobic conditions compared to aerobic conditions. The first biodegradation step can be initiated by either adding fumarate or carboxylation for n-alkanes, whereas, for aromatic compounds, this could be done via adding fumarate, carboxylation, or hydroxylation (Widdel & Rabus, 2001; Fuchs, et al., 2011; Head, et al., 2014). The anode not only serves as an alternate electron acceptor upon the biological mitigation of hydrocarbons (Lovley & Nevin, 2011; Viggi, et al., 2015), but also assists oxygen formation and pH alteration in the vicinity of the anode (Daghio, et al., 2017). The values of standard oxygen potential are known, however, in real-life applications, the overpotential of oxygen evolution relies on the material of the electrode (Anglada, et al., 2009). Oxygen liberation via oxidation of water is commonly concomitant with a corresponding drop in pH, which might have some impact on biological degradation. Some of the factors that determine the oxygen evolution potential and the treatment effectiveness are the type, design, and electrode material (Radjenovic & Sedlak, 2015). In a marine medium, particularly under anaerobic conditions, the electrochemical potential for oxygen evolution reaction can differ according to the characteristics of seawater (e.g., salinity, ionic forms, and concentration), the existence of further hydrophilic

components in the sediment, and ecological factors (e.g., temperature and pH) besides the electrode properties (Daghio, et al., 2017). The anaerobic zone can be supplied with oxygen by means of high potential electrodes, this oxygen can act as the electron acceptor for the bacteria for pollutants' biodegradation. An example is for the mineralization of cis-dichloroethene ( $85 \mu\text{mol/L}$ ), in contaminated groundwater, which was promoted by a polarized electrode made of graphite in the presence of ethene as a cometabolic substrate (Aulenta, et al., 2013).

Table 1. Major studies related to oxidation in BES for the remedy of oil spillage in marine sediments/sediment seawater

Compound/ mixture	Microorganism /mixed culture	Redox mediator	Working electrode material	Medium treated	Working electrode potential (vs. SHE)	Maximum current/power/voltage	Removal capability	Reference
Toluene	Mixed culture	Not detected	Unpolished graphite rod	Sediment seawater slurry (1:4)	+500 mV	N.A.	100% removal from 10 $\mu$ M	(Zhang, et al., 2010)
Benzene	Mixed culture	Not detected	Unpolished graphite rod	Sediment seawater slurry (1:4)	+500 mV	N.A.	100% removal from 9 $\mu$ M	(Zhang, et al., 2010)
Naphthalene	Mixed culture	Not detected	Unpolished graphite rod	Sediment seawater slurry (1:4)	+500 mV	N.A.	~100% removal within 9 days from 100 $\mu$ M	(Zhang, et al., 2010)
TPH	Mixed culture	Not detected	Stainless steel brush	Contaminated sediment	N.A.	190 mV		(Morris & Jin, 2012)
TPH	Mixed culture dominated by $\alpha$ - Proteobacteria, $\gamma$ - Proteobacteria and $\delta$ - Proteobacteria	Not detected	Graphite rod	Marine sediment	N.A.	N.A.	21 $\pm$ 1% removal within 200 days from 11.9 $\pm$ 0.12 g/kg	Viggi, et al., 2015)

## 1.7 How material and operational conditions affect BES

In the following text, the influence of material type and operational conditions on the performance of BES is presented.

### 1.7.1 Material of electrodes

Selecting a proper material for making the anode plays a crucial role in the efficacy and selectivity of biodegradation of hydrocarbons (Anglada, et al., 2009). It is necessary for the electrode material to be: (a) highly stable both physically and chemically, (b) highly conductive for electricity, (c) possessing high selectivity and catalytic activity for targeted pollutants, and (d) relatively cheap regarding its lifespan. (Daghio, et al., 2017). For the biodegradation of hydrocarbons, the use of affordable and durable materials is preferred. Material selection also determines the nature of oxidation reactions by acting either synergistically or competitively with oxygen evolution side reactions. It also primarily determines the oxygen evolution reaction overpotential (OER) in real applications (Anglada, et al., 2009). Furthermore, the surface roughness and surface morphology of the electrode influence the selection of the electrode and the degree of adhesion of microorganisms on the electrode surface (Li, et al., 2014); (Pocaznoi, et al., 2012); (Santoro, et al., 2017). The commonly utilized electrodes in MFCs encompass carbonaceous electrodes, i.e., carbon foam, mesh, rod, etc., and metallic substances such as stainless steel. All these materials are anti-corrosive and biocompatible (Ghangrekar & Neethu, 2020).

#### **Carbon materials**

Owing to their chemical inertness to microbes, large specific surface area (SSA), good conductivity, and comparatively low price, carbon materials have been often employed in MFCs as anodes (Dumitru & Scott, 2016). The following examples can be given: graphite in different shapes, i.e., rods, brushes, foams, sheets, etc. One of the major drawbacks of employing conventional carbon materials in MFCs is the clogging of their pores with the biofilm which in turn reduces their electrocatalytic activity (Dumitru & Scott, 2016). Moreover, it is worth noting that in some cases, the application of these carbonaceous materials in wide-scale MFCs is hindered, due to low specific area, flimsiness, expensiveness (Dumitru & Scott, 2016). It has been demonstrated that the surface characteristics and the affinity for microorganisms of the anode material are key determinants affecting the strength of electrical connection and attachment between the electrode from one side and microorganisms from the other side (Dumitru & Scott, 2016).

Nevertheless, these limitations can be overcome by using different surface modification methods allowing the practical implementation of these carbon-based materials (Dumitru & Scott, 2016).

### **Metal materials**

Thanks to the improved conductivity of metals over carbon-based materials, some metals were utilized as an anode of MFCs, although this use was somewhat limited because of the non-corrosive prerequisites of the MFC. Stainless steel and titanium are typical metals considered as acceptable anode potential in several researches. For instance, Copper was tested as an MFC anode (Prasad & Tripathi, 2017; Bian, et al., 2018). Copper proved to be unsuitable for this purpose as a result of its toxicity to microbes and susceptibility to corrosion, despite its superiority to carbonaceous materials with regard to current generation (Dumitru & Scott, 2016). Due to having high mechanical properties and its corrosion resistance, stainless steel has been investigated as both anode and cathode in MFCs which can provide a durable and long-lasting operation in comparison with carbonaceous electrodes (Dumitru & Scott, 2016; Papillon, et al., 2021). Dumas and colleagues tested stainless steel and it achieved good results as a cathode, whereas it gave lower power density when used as an anode (Dumas, et al., 2008). According to Erable and his research group, stainless steel was found to be a promising bioanode in combination with bacteria under potential control when a proper inoculation method is applied (Erable & Bergel, 2009). Titanium was also proposed as an MFC anode. It was analyzed by (ter Heijne, et al., 2008) together with graphite and platinum-coated titanium. Uncoated titanium did not pass any current. In addition, noble metals have been studied to be used as MFC anodes as they diminish the cell's internal resistance, however, the high cost and the poor bacterial attachment to their surfaces limited their large-scale implementation in MFC (Richter, et al., 2008; Torabiyani, et al., 2014; Yaqoob, et al., 2020; Tiquia-Arashiro & Pant, 2020). The smooth surface of stainless steel and titanium complicate the attachment of enriched bacteria on their surfaces besides the naturally formed nonconducting oxides. These limitations could be overcome by surface modification using different materials to enhance surface characteristics (Dumitru & Scott, 2016).

Many articles investigated the use of gold as an MFC anode owing to its higher conductivity and compatibility with microorganisms and the ability to use it in traditional micromanufacturing technologies (Qian, et al., 2009; Choi & Chae, 2013), however, this



cannot change the fact that gold is not a viable option for scaling up even when it is used as a coating in MFCs. The research trend to utilize metals as anodes in MFCs focuses on ameliorating the surface properties through modification with metal or metal oxide nanoparticles (Dumitru & Scott, 2016).

#### 1.7.2 Radius of influence

Before scaling up BES technologies for in-situ bioremediation, the aspect of how far the degradation zone will extend from the anode surface, i.e., the radius of influence (ROI), should be addressed. (Wang , et al., 2012) attempted to estimate the ROI when investigating saline soil for TPH biodegradation. The conductivity was 8.32, which was high enough to lower the electrical resistance, however, the improvement in the degradation rate was witnessed solely in samples taken from the anode's vicinity (less than 1 cm) 25 days after incubation, whereas at 2 - 3 cm away from the anode the removal rate was equivalent to the open-circuit control removal rate (Wang , et al., 2012).

It has been recently shown that the estimated ROI can extend from the electrode surface to a distance of 34 cm within 120 days. It was also noted that the mitigation of hydrocarbons was mainly in the proximity of the anode (1 cm away), however, with time, this effect increased (Daghio, et al., 2017). It was hypothesized that both the improved TPH elimination and the distance from the anode are linearly correlated. The authors predicted that when a BES with a radius of 7.5 cm is used, the ROI would extend to a distance of 90 cm in a 45-day period, which can be interpreted to a ratio of 12 between the ROI and the BES radius. However, for distances more than this, no experimental proof has been presented (Lu, et al., 2014). It was also posited by the same author that the increase in the BES radius is directly proportional to the increase in ROI, nevertheless, this needs to be investigated in field bioremediation.

### 1.8 The use of microorganisms in BES for the remedy of oil spillages

Zhang and coworkers were the first to inoculate iron-reducing bacteria MFC with hydrocarbons as a substrate. (Zhang, et al., 2010). In his study, it was demonstrated that *Geobacter metallireducens* were able to exploit a graphite electrode as an electron acceptor for toluene degradation. A previous study indicated that *Shewanella oneidensis* MR1 14063 has been used in a MFC in the degradation of phenanthrene (Adelaja, et al., 2014). In addition to obligate anaerobes, *Pseudomonas aeruginosa* NCTC 10662 has been reported to achieve quicker degradation of phenanthrene than *Shewanella oneidensis* ( $25.2 \mu\text{M d}^{-1}$ ) in an MFC under the same operating circumstances (Adelaja, et al., 2014). In other studies, the ability of aerobic and facultative anaerobic bacteria for hydrocarbons oxidation with the anode playing the role of electron acceptor has been demonstrated. Using self-produced shuttles as mediators, *Cupriavidus basilensis* efficiently degraded phenol by means of electron transfer with the anode (Friman, et al., 2013); whereas it was presumed that a catabolic intermediate, i.e., catechol, is observed in the bioelectrochemical degradation of toluene, under low oxygen concentration, via *Pseudomonas putida* F1 (Friman, et al., 2012). That discovery gives rise to questions related to the degradation mechanism and the contribution of oxygen in the biodegradation of hydrocarbons using *Pseudomonas* spp. using an anode (Daghio, et al., 2017). In fact, microbes from this genus are well defined as hydrocarbon degrading bacteria that are observed under aerobic conditions, and the mitigation of hydrocarbons initiates by adding hydroxyl groups with oxygenase catalyzing the reaction which necessitates molecular oxygen (Jindrová, et al., 2002). In studies related to the elimination of benzene from groundwater, it was suggested that the biodegradation of hydrocarbons started with catalysis by monooxygenase and succeeded by removing the intermediate anaerobically (Daghio, et al., 2017). The aforementioned discoveries assume their significance from the recent studies which consider hydrocarbon-containing environments to be anoxic and therefore involve anaerobic reactions which occur with no oxygen. The massive presence of typical aerobic bacteria (An, et al., 2013) in these anoxic environments was ascribed to several causes; (i) potential oxidation during sampling (ii) mixing with exogenous microbes (iii) diffusion of oxygen (iv) hypothetical pathway that generates reactive oxygen, such as the radiolysis of water (Daghio, et al., 2017). It can be also assumed that these aerobes and facultative anaerobes utilize electron acceptors in

several ways more than it has been realized so far. The traditional idea of *Pseudomonas* spp. using nitrate and other oxidized nitrogen species, in the absence of oxygen, might not reflect their versatility in using electron acceptors (Daghio, et al., 2017). *Pseudomonas aeruginosa* was reported to secrete phenazine electron shuttles which transfer electrons to the anode of a MFC (Rabaey, et al., 2005). The hydrocarbon activation pathway in anaerobic BES by microbes using molecular oxygen and necessitating mono- and di-oxygenase is still inscrutable, however, research has provided evidence for the presence of hydrocarbon activation pathways related to BES in articles discussing the methanogenic biodegradation of crude oil and alkanes. A study investigating a petroleum degrading consortium enriched with heavy produced water, which is a byproduct from oil and natural gas extraction (Berdugo-Clavijo & Gieg, 2014), reported the likelihood of *Pseudomonas* spp. of undergoing syntrophic interactions with methanogens, hence playing a role in both hydrocarbon fermentation and methanogenesis. They inoculated a microbial community consisting mainly of *Smithella* sp. which is an alkane fermenting microbe, together with acetoclastic and CO<sub>2</sub> reducing methanogens. The medium was anaerobic sand columns spiked with residual oil, anaerobically incubated for around 300 days. As a result, crude oil hydrocarbons degraded and methane was released. After analyzing the aforementioned microbial consortia, a significant variation in their composition was observed, with CO<sub>2</sub> reducing methanogens becoming the most abundant species in the consortium. The most noteworthy observation in this study was *Pseudomonas* spp. constituting the main bacterial taxon (Berdugo-Clavijo & Gieg, 2014). This study indicates that *Pseudomonas* spp. indeed contains metabolic mechanisms enabling alkane activation in anoxic conditions, providing that it is certainly amenable to alkane fermentation (Daghio, et al., 2017). Not only pure cultures were studied in BES for hydrocarbon degradation, but also mixed cultures. It was posited in a few articles that the recalcitrance of the substrate and the existence of alternate electron acceptors might influence the choice of the microfloral community. It was reported that when rapidly biodegradable substrates, i.e., acetate, are employed in BES systems, the main constituents of the microbial community that would form are iron-reducing bacteria (Daghio, et al., 2015; Zhu, et al., 2014; Kiely, et al., 2011). Nevertheless, their role seems to be minor in the biodegradation of hydrocarbons in BES. From this, it can be inferred that the complexity of the substrate induces different types of pathways at the expense of direct electron transfer (DET) (Rakoczy, et al., 2013; Lu, et al., 2014). It was reported by Morris and his research group in an analytical study of TPH-polluted soil that the most dominating

bacterial phylum after remediation was Proteobacteria (Morris, et al., 2009). Within that phylum, the most common were Beta- and Gammaproteobacteria, in those, *Bordetella*, *Pseudomonas*, and *Comamonas* were the dominating ones. Among those three genera, *Bordetella* was unreported as electroactive. This restates that when the contaminant is recalcitrant, the choice of a certain type of microbial community is influenced by other factors than the electron transferability to the anode (Daghio, et al., 2017). A number of modern researches drew attention to this finding, positing a major role for microbes participating in the sulfur cycle during BES, such as hydrocarbon degradation in marine sediments (Viggi, et al., 2015; Daghighi, et al., 2016).

### 1.9 The sulfur cycle effect

In seawater, sulfate is considered the second most abundant anion, with a concentration of approximately 29 mM (Algeo, et al., 2015). Sulfate was reported to promote the degradation of around 50 percent of the deposited organic pollutants in continental shelves (Jørgensen, 1982). The mineralization of hydrocarbons by sulfate reducers received attention owing to some studies that highlighted its potentiality in the oxidation of hydrocarbons in marine ecosystems (Daghio, et al., 2017).

Subsequent to the Deepwater Horizon oil spill calamity in April 2010, which is considered one of the most dreadful oil spill accidents in history, more research was conducted in bioremediation of marine environments (Roberts, et al., 2015; Martin, et al., 2020; Turner, et al., 2019). Kimes and coworkers were able to identify the metabolites and genes taking part in the anaerobic mineralization of hydrocarbons in a metagenomic study (Kimes, et al., 2013). The study unraveled the abundance of anaerobic hydrocarbon-degrading bacteria, such as *Desulfatibacillum alkenivorans* AK-01 which is a SRB (Sulfate reducing bacteria) belonging to the Deltaproteobacteria class (So & Young, 1999). Growing evidence corroborates the complexity of the role the sulfur cycle plays in sulfate-containing environments for crude oil degradation, acknowledging the participation of other microorganisms in conjunction with SBR in this degradation. For instance, Sherry and coworkers explored the degradation of crude oil in the prevalence of sulfate-reducing conditions, via the preparation of an anaerobic microcosm spiked with oil and incubated for 300 days (Sherry, et al., 2013). The results showed the mineralization of C7-C34 alkanes accompanied by the elimination of sulfate. The molecular analysis of 16S rRNA genes of the samples detected sequences from Gammaproteobacteria class intimately linked to

*Marinobacterium* sp. and others from the Peptostreptococcaceae family among the Firmicutes phyla instead of the ordinary SRB (Sherry, et al., 2013). Firmicutes have also been the most frequently identified, followed by the Gamma-, Delta and Epsilon Proteobacteria, based on extensive research of the microfloral population data from a variety of crude oil and hydrocarbon-contaminated anaerobic media (Gray, et al., 2010). In a further analysis conducted on petroleum reservoirs, where only microbial communities were the subject of attention, Firmicutes accompanied by Gamma-, Epsilon- and Deltaproteobacteria were the commonest findings (Hubert, et al., 2012). Zopfi and his research group reported that sulfur compounds with intermediate oxidation states led to the reoxidation of sulfide stemming from the reduction of sulfate by means of intermediate sulfur species (Zopfi, et al., 2004). Figure.6 illustrates several interactions involved in the sulfur cycle.

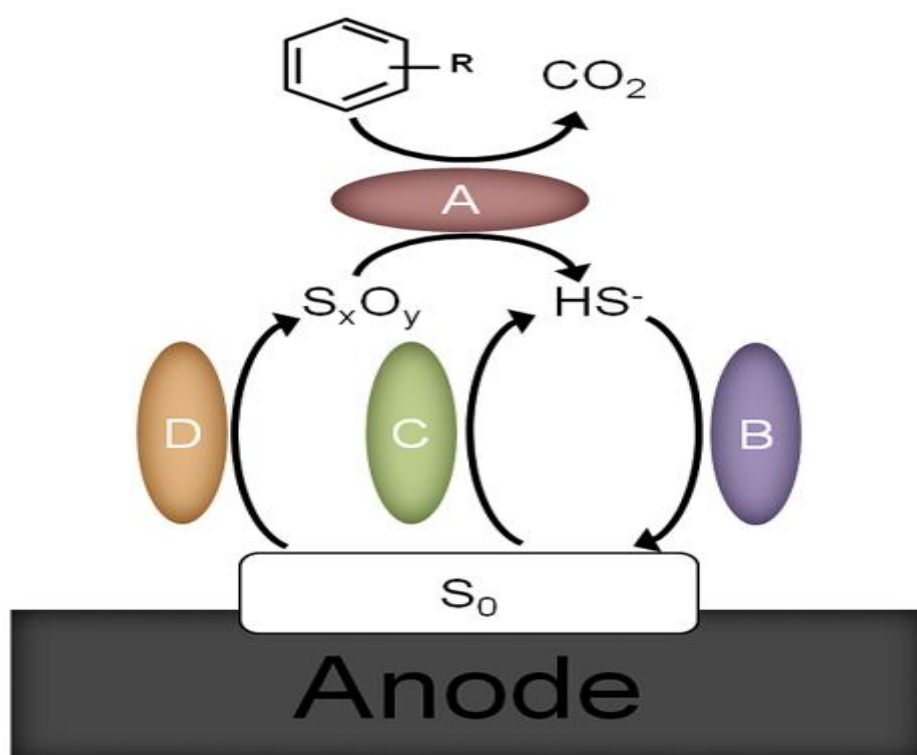


Figure 6 - Possibilities of the role that sulfur cycle plays in hydrocarbon degradation: (i) oxidation of hydrocarbons and reduction of  $S_xO_y$  to  $HS^-$  via sulfate reducers (ii) Oxidation of  $HS^-$  on the anode. (iii) Reduction of  $S_0$  to  $HS^-$  (iv)  $S_0$  oxidation to  $S_xO_y$  (Daghio, et al., 2017)

In a study investigating the bioelectrochemical degradation of toluene, it was shown that Desulfobulbaceae and Desulfobacteraceae had the superiority over other microbial communities growing in polluted marine sediment, because of the presence of hydrocarbons, represented in toluene, together with a synergistic action from the anode and sulfate ions

acting as electron acceptors (Daghio, et al., 2017). Simultaneously, under anaerobic conditions, reduction of sulfate occurs with a possibility to be oxidized to sulfur on the anode via either abiotic or biotic process which is likely due to the enrichment of Desulfobulbaceae family (Daghio, et al., 2017). The interesting fact is that family contains some members belonging to filamentous bacteria known as cable bacteria, which grow in different kinds of sediments and are able to generate current over long distances via the oxidation of sulfide (Geelhoed, et al., 2020). Sulfur oxidation to sulfate is another possible pathway that can continuously supplement SRB with electron acceptors (Zhang, et al., 2014). In the aforementioned processes, the transfer of electrons from the microbes to the electrode can be achieved by sulfide ions acting as electron shuttles (Rakoczy, et al., 2013; Dutta, et al., 2009). These discoveries expand the potential of BES. One additional advantage of the sulfur cycle in hydrocarbon biodegradation in BES is lowering the toxicity thanks to the sulfide oxidation to sulfur and then to sulfate (Dutta, et al., 2008; Zhang, et al., 2014).

#### 1.10 Advantages and drawbacks of bioelectrochemical processes

BES methods to promote in situ bioremediation have many advantages. One of their major merits is the possibility of controlling the conversion of pollutants by altering anode or cathode potential (Daghio, et al., 2017). Making the reaction thermodynamically feasible and acclimatizing to the in-situ conditions can be achieved by setting energy levels (Daghio, et al., 2017). Additionally, the flow of electrons could be preserved and stabilized for longer durations (Daghio, et al., 2017). The ability to keep a steady flow of electrons for appreciable periods without the need to continuously supply electron donors/acceptors (Morris & Jin, 2008). Moreover, as adding chemicals is needless, the need and costs of transportation and storage are phased out. To sum up, bioelectrochemical remediation is superior to other conventional remediation techniques for being greener and more cost-effective (Aulenta, et al., 2009). Concerning operation expenses of an in-situ bioremediation plant, it is worthy to mention that as experimental data from real applications are lacking, it is challenging to fully assess BES cost, however; it can be logically assumed that the major cost, in that case, will be dedicated for the electrode material (Daghio, et al., 2017). Also, this assumption should consider the cost per lifetime of the material. Another benefit of BES techniques is their selectivity over physicochemical ones, which might produce higher toxic by-products than the targeted pollutants (Daghio, et al., 2017). For instance, in one study, a biocatalyzed cathode was used to convert nitrobenzene to aniline (which has less toxicity and is readily

degradable) at 99% efficiency (Wang, et al., 2011). On the other hand, when an electrochemical approach was applied, nitrosobenzene, which is highly toxic compared to aniline, was formed (Mu, et al., 2009). The process can be more efficient when graphite/other carbonaceous materials are employed due to carbon's absorbability, hence, making the contaminant more vulnerable to the biodegradation zone through the anode and biocatalyst interaction (Rabaey & Keller, 2008; Zhang, et al., 2010).

BES methods may have some shortcomings. The major drawback is the slowness of anaerobic degradation compared to the anaerobic one which involves a more effective enzymatic activation via oxygenase throughout the primary step of the biological pathway (Daghio, et al., 2017). Moreover, previous studies showed that while MFC is promoting the degradation of organic pollutants, its efficiency might be influenced by the rate of oxidation at the anode and the kinetics at the cathode (Liu & Logan, 2004; Zhao, et al., 2006). In contrast, it is of particular concern to avoid the generation of chlorine gas (when MEC is employed in a marine environment) which exceeds the targeted pollutant in toxicity and is able to react with all inorganic and organic compounds to form noxious products (Richardson, et al., 2007). Besides, some factors should be considered, including the choosing suitable materials, the potential and the influence of the ROI, and the encountered restrictions toward scaling up the technology should not be ignored in the economic analysis of electrobioremediation.

### 1.11 The Microbial Electrochemical Snorkel (MES)

MES can be considered as the simplest model of a MFC as it is constructed by joining a bioanode with a cathode, which can be biotic or abiotic (Erable, et al., 2011), in a short-circuited structure (Santoro, et al., 2017). Being a short-circuited MFC means that the voltage between the anode the cathode is zero, which allows obtaining the maximum possible current from that system (Hoareau, et al., 2019)

In contrast to ordinary MFCs, it does not generate power and that is why it is known for the simplicity of its design, as it does not require any membranes or separators, solely one rod of conductive material that extends two segregated zones differing in the chemical composition. For instance, a buried rod is in an anaerobic sediment while the upper part is in the oxic water (Fig.7) (Hoareau, et al., 2019).

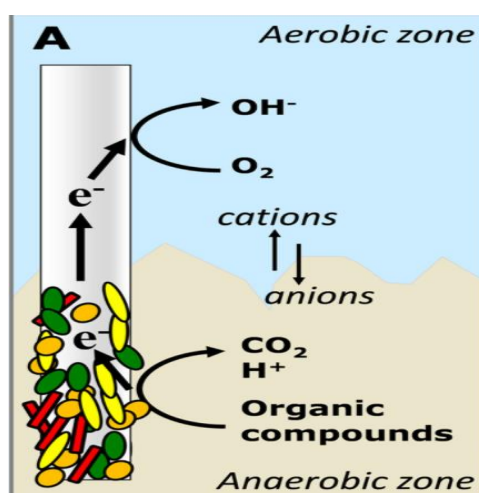


Figure 7 - An illustrative figure for the MES (Hoareau, et al., 2019)

A thin film of electroactive bacteria is formed on the buried part of the snorkel, releasing electrons to the upper oxic region where they result in the formation of water. This simplicity of the design and process guarantees low-cost and low maintenance of the system (Hoareau, et al., 2019). MES can be an ideal solution when the aim is to detoxify organic pollutants by speeding up the rate of chemical reactions at the expense of generating power (Hoareau, et al., 2019). This can be applied to several applications such as the biodegradation of sediments or soils (Wu, et al., 2018), effluent cleanup (Yan, et al., 2019), and metal recovery (Dominguez-Benetton, et al., 2018). A MES can be considered an imitating process of cable bacteria (Matturro, et al., 2017; Pfeffer, et al., 2012). Having a similar method of operation to cable bacteria makes the MES more feasible to be scaled up (Hoareau, et al., 2019). A myriad of applications can benefit from MES, such as bioremediation of crude oil-polluted sediments (Viggi, et al., 2015), wastewater treatment (Erable, et al., 2011; Aguirre-Sierra, et al., 2016), and the abatement of sulfate (Viggi, et al., 2017) or nitrate (Yang, et al., 2015) according to the literature. Another benefit that has been postulated for MES that it could inhibit the generation of methane under anoxygenic conditions via deflecting the flow of electrons (Lovley, 2011). The MES also can contribute to tackling other ecological issues, such as the formation of sulfide and the conversion of mercury to methyl mercury (a highly neurotoxic compound) (Lovley, 2011).



## 1.12 MES applications

MES was in the center of attention of several research work for the abatement of organic and inorganic contaminants. In the following text a brief description for these previous efforts is introduced.

### 1.12.1 Hydrocarbon biodegradation

The application of MES has been extended to the remediation of marine sediments contaminated with oil-spill hydrocarbons. Microcosms were prepared with one or three snorkels inside, together with control samples, snorkel-free or autoclaved + snorkel (Viggi, et al., 2015). The snorkel was a basic rod made of graphite, embedded in a fuel oil - spiked sediment with the cathodic part in the overlaying oxic water (Viggi, et al., 2015). After 200 days of incubation, the snorkel-free control did not show any change, however, the result was promising for the samples having one and three snorkels, attaining a decline in TPHs of 12 and 21% respectively (Viggi, et al., 2015). The result was very similar for all samples by the end of the incubation period after 417 days (Viggi, et al., 2015). The final result underscored the faster degradation rate attained by the snorkel with a low cost compared to conventional methods (Hoareau, et al., 2019). In another similar study undertaken by the same author, but this time on river sediment polluted with crude oil, the chemical and biological reactions taking place in the presence of MES were modeled (Viggi, et al., 2017). MES led to the degradation of TPHs in the sediment, which in turn expedited sulfate reduction (Viggi, et al., 2017). Both MES and the marine sediment had similar microbial communities with Proteobacteria as the major phylum (Matturro, et al., 2017). The MES surface was mainly colonized by Alphaproteobacteria, which include sulfur and sulfide oxidizing bacteria that possibly could transfer the generated electrons from sulfide oxidation, whereas deltaproteobacteria were mainly in the sediment (Matturro, et al., 2017). The MES was presumed to influence the microorganisms on its surface and others away from it and of promoting microbial degradation via sulfate reducers in its proximity (Mapelli, et al., 2017).

### 1.12.2 The degradation of organic matter in wastewater treatment

Erable and coworkers studied MES performance in COD reduction, by testing it in comparison to a regular MFC, the snorkel outcompeted the MFC with a 60 - 75% reduction to only 40 - 50% from the MFC (Erable, et al., 2011). MES has achieved remarkable success when employed in a constructed wetland (Ramírez-Vargas, et al., 2018). It was implemented as a sort of electroconductive biofilter incorporated in an artificial lagoon and compared to

a traditional lagoon provided with gravel (Aguirre-Sierra, et al., 2016). The result was in favor of MES with 3 and 4.5 folds saving in time for both COD and BOD<sub>5</sub> removal respectively (Aguirre-Sierra, et al., 2016). MES was used in pig manure treatment, in comparison to a biosand filter, achieving 90% COD reduction to only 81% for the filter (Ramírez-Vargas, et al., 2019).

#### 1.12.3 Nitrate removal

In a study conducted by Yang and coworkers for denitrification, MES was used by immersing carbon felt in the sediment as an anode, and iron cylinder in the solution containing NaNO<sub>3</sub>, as a cathode. As a result, organic matter was oxidized at the anode surface and NO<sub>3</sub> was reduced to N<sub>2</sub> at the cathode surface. (Yang, et al., 2015). The MES attained 98% efficiency in denitrification in 16 days. It acted as a collector for the electrons and nitrates in the sediment and the oxygenated solution, respectively (Yang, et al., 2015).

#### 1.12.4 Soil bioremediation

When soil is oversaturated with water, it is called ‘‘flooded soil’’. This condition creates a shortage of electron acceptors which in turn could restrict the microbial degradation efficacy of organic contaminants. This situation can be tackled by traditional bioremediation methods via providing electron acceptors, such as O<sub>2</sub> and NO<sub>3</sub> (Yu, et al., 2014), which unfortunately adds more cost and generates harmful by-products (such as NO<sub>2</sub>) (Pandey & Fulekar, 2012). Another way to supply electrons is by using an electrochemical-assisted bioremediation technique, called bioelectroventing, which has been compared in a recent study with other bioelectrochemical approaches, such as MES for treating soils contaminated with atrazine (herbicide), to fully mineralize <sup>14</sup>C-atrazine to readily degradable CO<sub>2</sub> (Domínguez-Garay , et al., 2014). Graphite felt electrode was used as the MES, vertically immersed in the soil, and part of it extended to the overlaying water. By the end of the treatment after 20 days, there was an 80% drop in soil toxicity in the MES while it was only 55% in the control treatments (Domínguez-Garay , et al., 2014).

#### 1.12.5 Metal recovery

It has been recently proved by Mitov and coworkers, that MES is capable of reclaiming copper from an aqueous solution (Mitov, et al., 2021). Two MES were prepared by short-circuiting copper electrodes with bioanodes of a MFC, (one with a membrane, and the other without) and compared with a regular MFC. It took the MES without membrane to achieve 95% copper recovery in only 2 days, while MES with membrane and the MFC achieved

slightly higher recovery percentages of  $97.8 \pm 4.5\%$  and  $98.3 \pm 4.8\%$  respectively in 10 days (Mitov, et al., 2021). Copper oxide accumulated on the cathodes. Additionally, the MES without membrane was the highest in terms of cathodic efficiency with  $61.7 \pm 6.9\%$ . Moreover, MES achieved a 10% higher copper recovery than the regular MFC (Mitov, et al., 2021).

## 2 THE ROLE OF ADDITIVE MANUFACTURING IN THIS STUDY

The electrodes used herein were manufactured by powder bed fusion (PBF) which is one technology among many other types of additive manufacturing (AM), which is famously termed as 3D printing. AM is generally based on the concept of building up material layers one over each other rather than removing them. It has numerous benefits such as the possibility to produce intricate designs and it does not cause material wastage which is common in traditional manufacturing methods, as it is capable of reusing the unused powders, resins, etc. during the manufacturing process and the ability to produce different geometrical surface patterns; however, it still has some disadvantages like the slow pace and high cost of the process. (Ford & Despeisse, 2016; Bacciaglia, et al., 2019). Fortunately, AM is rapidly overcoming some of its early constraints, expanding its implementation across a wide range of industrial segments. Some of AM techniques are, material jetting, material extrusion, sheet lamination, directed energy deposition, and powder bed fusion with the latter being the most used one (King, et al., 2015). In this process, a laser or electron beam fuses the spread powder layers and melt them to form a solid material layer by layer until a 3D object is finally created (Bhavar, et al., 2017) as shown in Fig. 8.

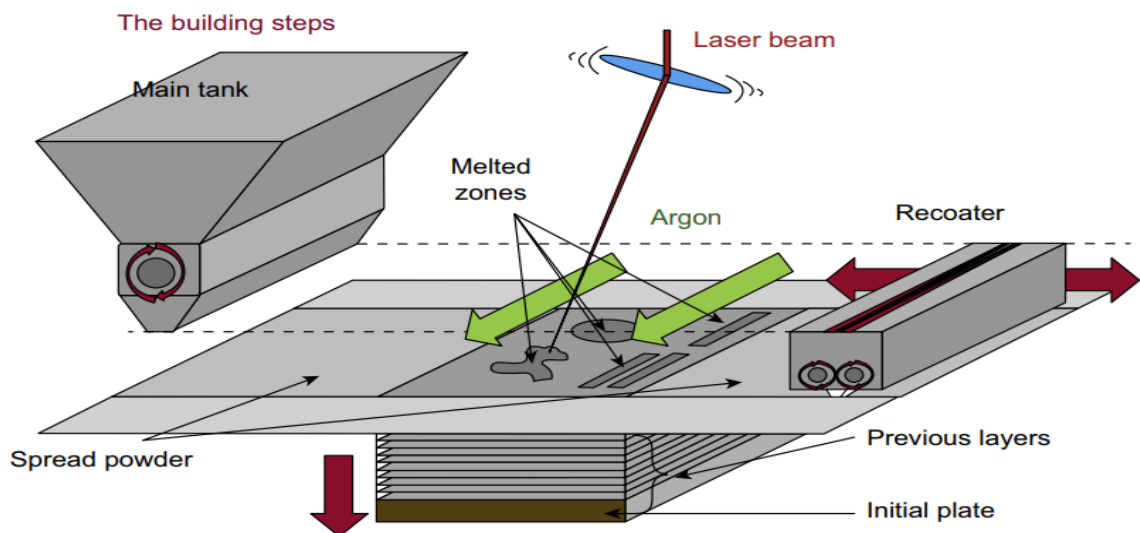


Figure 8. Schematic overview for laser-based powder bed fusion technology (Sun, et al., 2017).

The powder used in manufacturing the electrodes is the superalloy Inconel 718 nickel-based alloy which was chosen as an alternative to stainless steel SMO 254 grade that could not be found as a powder for additive manufacturing. Generally, Inconel® is classified as a superalloy because it is resistant to corrosion and is fully relevant for elevated pressure and heat environments. Superalloys have a plethora of high-temperature applications, including aircraft components, petrochemical and chemical plant equipment. Superalloys are also extensively used in the aviation and marine industries (Akca & Gursel, 2015). Interestingly, scrap metallic parts of Inconel 718, in particular, were used by (Lefebvre, et al., 2013) as the cathode in a MFC for wastewater treatment. Compared to W/Co alloy, Carpenter alloy, and Cu/Ni alloy, and after 1 year of service, Inconel 718's performance did not deteriorate and without detecting any Fe in the treated effluent, indicating no sign of corrosion and demonstrating the material's long-term endurance. In another study, Al Fozan and Malik studied the impact of seawater level in three water zones on several alloys widely used in marine environments such as 304 SS, 316L SS, 90/10 Cu/Ni, 70/30 Cu/Ni, Ni-based alloys (Incoloy 825 and Inconel 625) together with carbon steels (G1010 and 1020. Inconel 615 (which belongs to the same family as Inconel 718) had the lowest corrosion rate at all three locations (Al-Fozan & Malik, 2008). In this work, the used technology-enabled surface patterning of one set of the electrodes, i.e., to expand the specific surface area of the electrodes, and the goal from this was to investigate its impact on the rate of TPH biodegradation in marine sediment compared to the flat electrodes used.

### 3 EXPERIMENTAL PART

#### 3.1 Aim of the experimental part

The main objective of the experimental part was to develop a novel bioelectrochemical remediation technology based on the concept of “Microbial Electrochemical Snorkel” (MES) that can aid in the enhancement of hydrocarbon biodegradation in subsurface layers of aquatic environments.

The electrodes used in this experiment, i.e., snorkels, were fabricated via additive manufacturing, in particular, via selective laser manufacturing (SLM). The material of the used powder was Inconel 718 superalloy as an alternative to the famously used Stainless Steel 254SMO grade which couldn't be found as a powder for this technology. Nickel-based alloys have superior corrosion resistance in a range of environments including seawater (Al-Fozan & Malik, 2008; Popovich, et al., 2015).

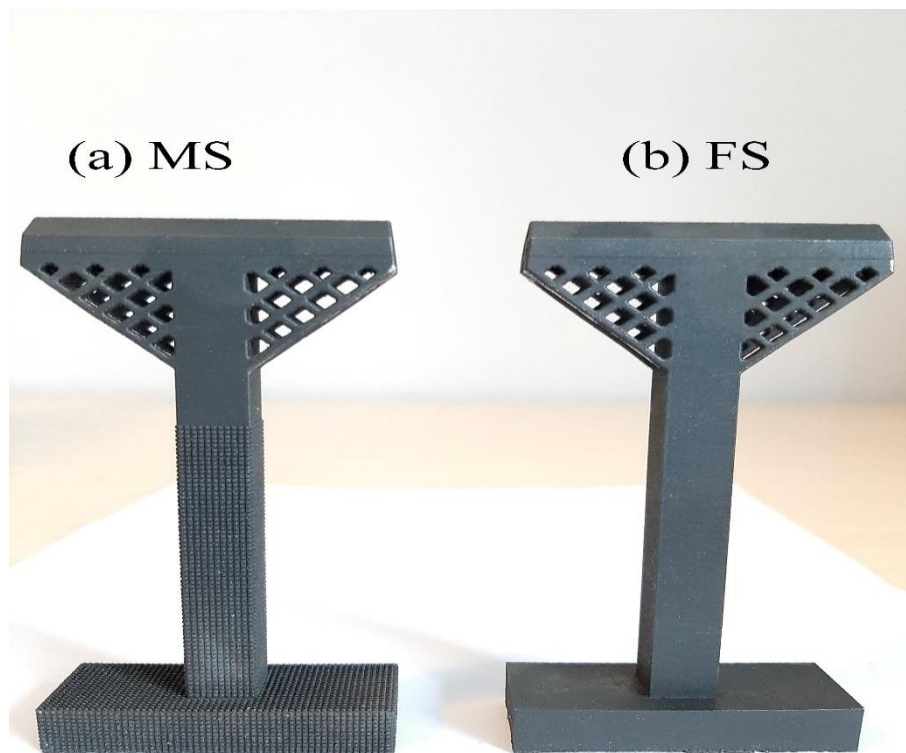


Figure 9. (a) Snorkel made with microstructured surface (MS), (b) Snorkel with flat surface (FS)

The idea was to manufacture the snorkel in the form of two horizontal structures connected with one vertical structure that extends through the oxic and non-oxic regions. More surface area was allocated to the lower horizontal part of the electrode, i.e., the anode, to provide more space for the biofilm formation and see how this may impact the rate of the TPH degradation. A micropillar surface patterning was selected as it was proved to significantly enhance the biofilm formation by allowing the cells to grow in a quieter environment, unlike smooth surfaces (as in the case of the second electrode) which may decelerate the biofilm formation (Champigneux, et al., 2018). The smooth surface electrode has been used for comparison. (Fig.8).

### 3.2 Experimental microcosm setup

In this work, sacrificial microcosms have been prepared using marine sediment sampled from the Baltic Sea, Porvoo site, near the refinery, and petrochemical industry treated wastewater outlet (Miettinen, et al., 2019). Marine fuel oil (Teboil RMD 80 0,1) was used to artificially contaminate the sediment.

The sediment was divided into four quarters and one of these was amended with fuel oil previously mixed with hexane and placed in the sample bottles. This mixture was left 24 hours under a fume hood to allow the hexane to evaporate. Afterward, the three remaining quarters of the sediment were equally distributed into the sample bottles and mixed all together with the oil-spiked quarter to homogenize the whole samples before adding the other ingredients. The final concentration of the oil in the sediment was approximately 38 g/kg sediment.

P.S: Due to the non-availability of inert atmosphere equipment which was needed to preserve the anaerobic microbial community in the sediment, aerobic mixing was performed. The sample ingredients were placed in a 500 mL glass bottle ascendingly in the following order: oil-contaminated sediment (658 g), granular activated carbon GAC (20 g), seawater from the sediment collection site (234 mL). The snorkel was placed in the middle of the bottle extending from the top to the bottom of the bottle, to electrochemically connect the anoxic sediment and the superjacent oxygenated seawater. The total height of the snorkel is 11 cm, the width and thickness of the lower horizontal structures are 5 and 2 cm respectively, and the width of the middle structure is 1 cm. Owing to some limitations in the AM manufacturing method used, the electrode was designed in the form of a rectangular prism in the bottom and perforated an equilateral triangle on top instead of cylinders as was

planned. The perforation and the triangle were mainly an AM prerequisite in order to obtain a model more akin to the theoretical one. Approximately 70% of the total height of the snorkel was immersed in the sediment and 30% was in the oxic water.

There were two types of microcosms in the experiment; one containing a micro-structured snorkel and another containing a flat snorkel. The two microcosms' categories were given the following acronyms; MS (Micro-structured Snorkel) and FS (Flat Snorkel). To statically incubate the microcosms, a Forilabo® Bio Refrigerated Expert incubator was utilized and the incubation temperature was  $10 \pm 1^\circ\text{C}$ .



Figure 10. Experimental microcosms in the incubator.

At predetermined intervals, one bottle from each treatment was taken for analysis. The TPH in the sediment were analyzed via GC-FID after solid and liquid phases separation. IC (Ion Chromatography) was used to analyze the liquid phase, i.e., the oxic seawater for measuring sulfate anions (i.e., sulfate)

### 3.3 Analytical Methods

The liquid phase anions (i.e., sulfate) in the overlying seawater were quantified through the injection of a filtered sample ( $0.45 \mu\text{m}$  porosity) into an IC (IonPac AS22 analytical column, Thermo Scientific Dionex ICS-1100 system, Dionex Corp., Sunnyvale, CA, USA).

The total petroleum hydrocarbons (TPH) in the sediment were determined by GC-FID, upon liquid-solid extraction. Succinctly, a duplicate sediment sample ( $\sim 0.2 \text{ g}$ ) from each bottle



was dried with nitrogen gas and extracted with Heidolph Promax 2020 shaker (Heidolph Instruments GmbH & Co. KG) using pentane. Twelve samples of the sediment containing approximately 0.2 g in each, were placed in twenty-four tubes (two replicates per sample). 2 ml of pentane were added into each tube and was well shaken. The shaking took 45 minutes at a maximum speed of 400 rpm. After shaking, 1 mg of Florisil® was added to each sample to eliminate non-petroleum and polar components in the samples to be ready for GC-FID, using Agilent 7890 GC gas chromatograph. 2 ml from each tube were placed in screw-top vials on the GC autosampler. TPH was calculated by adding the separate peak areas using dodecane as a marker.

## 4 RESULTS AND DISCUSSION

### 4.1 Sulfate consumption

The original seawater used in the samples was initially analyzed at the beginning of the incubation on day 0 and then on day 104 for the incubated microcosms of all treatments (Fig. 11), where the main focus was on the quantification of sulfate anions, which perform a significant respiratory electron-accepting role in anoxic marine environments.

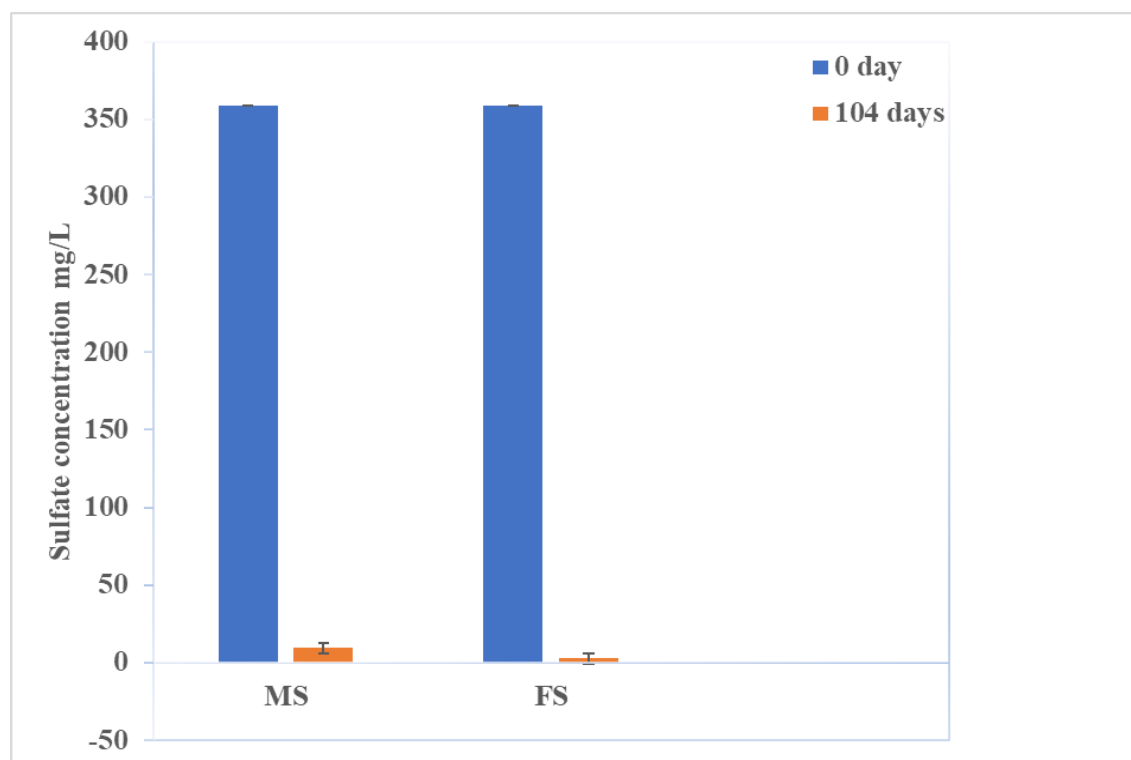


Figure 11. Sulfate concentration in the samples when evaluated at 0 and 104 days of incubation. Error bars signify the standard error of the replicate samples.

In the two types of microcosms, the sulfate concentration significantly dropped in the ratios of 97.3 and 99.2 for MS and FS respectively. The less reduction rate in MS compared to FS might be attributed to the microstructure in MS which could have an impact on making the snorkel more preferentially used over sulfate as an electron acceptor via providing a quieter environment for the biofilm formation which may consequently enhance the respiration of the bacteria. This assumption is in line with the fact that oxygen has higher redox potential than sulfate (+0.82 V compared to -0.217 V) which means that oxygen would be a

preferential sink for electrons than sulfate, however, this idea will be more supported in the TPH analysis.

#### 4.2 Total Petroleum Hydrocarbons (TPH) Degradation

The microcosms were analyzed on days 0 and 104 for the quantification of TPH to find out any potential change within that period in all incubated microcosms for all treatment (Fig. 12).

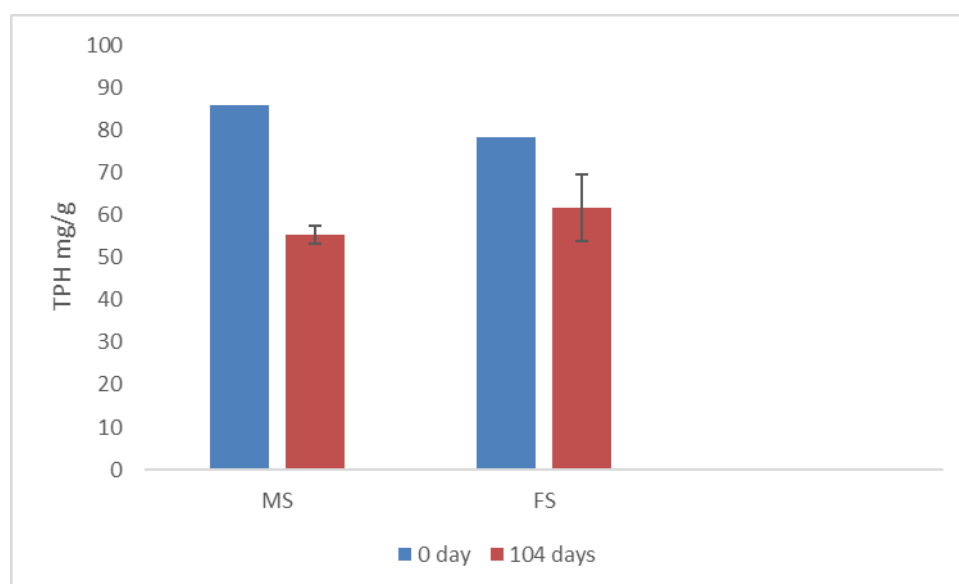


Figure 12. TPH concentration in the samples when evaluated at 0 and 104 days of incubation. Error bars signify the standard error of the replicate samples.

At the end of 104 days of incubation, the samples were analyzed by GC-FID for the quantification of TPH. A substantial elimination in the original TPH concentration was detected in the MS and FS samples in 35.45% and 21.26% respectively. This observation gives another indication that surface patterning may enhance the efficiency of the snorkel in the bioremediation of oil-polluted marine sediments compared to the flat-surface snorkel. It is also worth mentioning that this study confirms the consistency of these observations with prior microcosm studies conducted on sediments from different geographical sites with different types of marine fuel oils (Viggi, et al., 2015; Viggi, et al., 2017).

## 5 CONCLUSIONS AND FUTURE RESEARCH

This study demonstrated proof that a semi-buried electrode extending between two separated zones, i.e., oxic and anoxic, could boost the microbial degradation of PHs in benthic sediments, and this effect could be direct or indirect. This enhancement was witnessed in the discernible decline in TPH and sulfate concentration during the incubation period of 104 days, however, more research should be conducted on certain points to give more value to this work, for instance, in the literature, a snorkel made of graphite has been studied which was more affordable but lacking surface patterning and not viable in case of real-field applications due to poor mechanical properties, however, it would be interesting to make a comparison between these two to compare the degradation potential of graphite with a superalloy such as Inconel 718. Moreover, microscopy imaging technique/s should be employed to visualize the biofilm formation on the two types of electrodes used to study the potential enhancement in TPH degradation due to the surface patterning and increasing the specific surface area. Additionally, more research should be carried out to examine the impact of the radius of influence on the snorkel, by taking samples from measured distances from the snorkels to determine how far the degradation zone will extend from the anode surface which is extremely significant in scaling up the snorkel for real field application. Conducting molecular analysis would also valorize this study by unraveling the change in the microbial community in the sediment and on the snorkel during the incubation period which could further lead to the identification of the biochemical pathways governing the overall degradation process.

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