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Bioelectrochemical system for oil pollution remediation in marine sediments

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ABSTRACT

Bioelectrochemical system for oil pollution remediation in marine sediments

Among the many environmental challenges, the management, and remediation of contaminated marine sediments is perhaps the most difficult. A limited number of biological remediation methods are currently used to treat oil-polluted sediments. Bioelectrochemical systems (BES) to enhance the microbial oxidation of organic pollutants in marine sediments are extremely appealing since they can potentially serve as permanent, low-cost, and low-maintenance solutions. The aim of the current study was to assess the efficacy of BES in the treatment of several types of oil-polluted Baltic Sea sediments. In addition to the oil biodegradation efficiency, the changes in microbial community abundance, phylogenetic and functional structure in BES units were monitored using quantitative PCR and amplicon-based sequencing. The BES units consisted of a single chamber containing 300g of sediment and graphite-based electrodes (anode at the bottom and cathode on the sediment surface). The sediment samples were obtained from the Baltic Sea anoxic zone and spiked with crude oil (5% w/w), marine fuel IFO 180 (5% w/w), and crude oil with dispersant Finasol 52. During the 34-day experiment, the highest removal rate (45%) was recorded for BES units treating crude oil with dispersant. IFO 180 and crude oil removal were lower (32% and 28%, respectively). The voltage dynamics were similar in BES units with crude oil and IFO 180 increasing slowly during the first ten days of the experiment and staying in a steady state after that. The maximum power output (30.6 μW) was recorded in the BES unit with crude oil and dispersant. The abundance of bacteria was slightly increased in IFO180 BES units, while in the case of dispersant addition to crude oil, this value decreased twice. Dispersant addition also resulted in the decrease of archaeal abundance. The bacterial community structure substantially changed in the BES units during the experiment. The bacterial community structure was most strongly altered in the BES unit with dispersant. The study results suggest that the bioelectrochemical system-based treatment technology has good potential for the removal of oil compounds from heavily polluted marine sediments.

Keywords: bioelectrochemical system, microbial fuel cell, microbial community, total petroleum hydrocarbon, dispersant, bioremediation.

CERCS: T490

RESÜMEE

Bioelektrokeemiline süsteem naftareostuse eemaldamiseks meresetetes

Paljude keskkonnaprobleemide hulgas on saastunud meresetete puhastamine üks võib-olla kõige keerulisemaid probleeme. Nafta ja naftasaadustega saastatud setete puhastamiseks kasutatakse praegu piiratud arvu bioloogilisi tervendamismeetodeid. Bioelektrokeemiliste süsteemide (BES) kasutamine orgaaniliste saasteainete biolagundamiseks meresetetes on seni vähe uuritud valdkond. Käesoleva uuringu eesmärk oli hinnata BES-i efektiivsust erinevat tüüpi naftareostusega Läänemere setete puhastamiseks. Lisaks õli biolagunemise tõhususele jälgiti BES-s muutusi mikroobikoosluste arvukuses ja struktuuris kasutades kvantitatiivset polümeraasi ahelreaktsiooni ja 16S rDNA järjestamist. BES koosnesid ühest kambrist, mis sisaldas 300 g setet ja grafiidil põhinevaid elektroode (anood põhjas ja katood sette pinnal). Setteproovid saadi Läänemere anoksilisest tsoonist ja settele lisati toornaftat (5%), laevakütust IFO 180 (5%) ja toornaftat koos dispersandiga Finasol 52. 34-päevase katse käigus leiti suurim puhastusefektiivsus (45%) toornaftat dispergendiga töötlevate BES puhul. BES-i väljundvõimsuse dünaamika oli sarnane toornafta ja IFO 180 puhul kasvades aeglaselt esimese kümne päeva jooksul ja olles püsivas olekus pärast seda. Maksimaalne võimsus (30,6 μW) registreeriti BES-s toornafta ja dispersandiga. Bakterite arvukus IFO180 BES ühikutes veidi suurenes, samal ajal kui toornaftale lisatud dispergeeriva aine puhul vähenes see kaks korda. Dispersandi lisamisega kaasnes arhede arvukuse vähenemine. Bakterikoosluse struktuur muutus eksperimendi käigus oluliselt kõigis BES-i variantides. Uuringu tulemused näitavad, et bioelektrokeemilisel süsteemil põhineval lahendusel on hea potentsiaal naftaühendite eemaldamiseks tugevalt reostunud meresetetest.

Võtmesõnad: bioelektrokeemiline süsteem, mikroobne kütuseelement, mikroobikooslus, nafta reostus, biotervendamine.

CERCS: T490

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ABBREVIATIONS

1,2-DCA	1,2- dichloroethane
BES	Bioelectrochemical System
BOD	Biochemical Oxygen Demand
BTEX	Benzene, Toluene, Ethylbenzene, Xylenes
CEM	Cation Exchange Membrane
CO	Crude Oil
CO+D	Crude Oil + Dispersant (Finasol 52)
COD	Chemical Oxygen Demand
DWH	Deep Water Horizon
EGB	Electrogenic Bacteria
EIA	Environmental Impact Assessment
GC-FID	Gas Chromatography with Flame Ionization Detection
HC	Hydrocarbon
IFO	Intermediate Fuel Oil
MEC	Microbial Electrolysis Cell
MFC	Microbial Fuel Cell
MSS	Modular Slurry System
NMFS	National Marine Fisheries Service
OER	Oxygen Evolution Reaction
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polycyclic Biphenyls
PEM	Proton Exchange Membrane
PHC	Petroleum Hydrocarbon
REDOX	Reduction-Oxidation Reaction
SSA	Specific Surface Area
TEA	Terminal Electron Acceptor
TPH	Total Petroleum Hydrocarbon

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1 INTRODUCTION

Marine oil spills are defined as the inadvertent or intentional discharge of oil into the environment (water bodies) and to a degree exceeding 100 thousand gallons - this level of the spill is referred to as a "Major oil spill" (Dhaka & Chattopadhyay, 2021). Crude oil is a mixture of chemicals in thousands comprising aromatic and aliphatic hydrocarbons. Contamination by these chemicals, whether intentional or unintentional, brings about untold ecological repercussions. The sources of petroleum hydrocarbon pollution occur as a result of spills from anthropogenic activities ranging from the transport of crude and crude products, storing, drilling methods, manufacturing processes, and natural seeps (Gong *et al*., 2014). Immediately after the release of petroleum hydrocarbon, the soil in the underlying background in the ocean/seafloor stands at the receiving end and we may refer to the soil underneath the ocean/sea beds as the final consumer of these harmful chemicals products. They absorb these chemicals and owing to the absence of oxygen deep down in the ocean floors; a term referred to as an anoxic condition, there tends to be a slow level of biodegradation of these chemical pollutants as compared to oxic conditions as such these pollutants lie low forming major sinks as "Sediments" and great sources of environmental contamination to the vast marine ecosystem and ultimately humans. Sequel to the contamination of the vast marine ecosystem, the death of thousands of benthic organisms has been reported (Lynne Corn & Copeland 2011). The deepwater horizon disaster took a whopping 61 billion US dollars (Li *et al*., 2016) of which 5.5 billion US dollars were lost in fishing and tourism (Hagerty *et al*., 2010).

Hence at this point, it is important to note that two major goals are primary. First is the goal of preventing the oil spill in the first place which is somewhat impossible at this time and dispensation even with countless laws and legislation including sanctions. Secondly, is the prime goal to control the already spilled hydrocarbons by way of remediation technologies. These technologies can be either physical, chemical, or biological (bioremediation) and can as well be used in combination Also, a notable issue worthy of note is sediment mobilization, hence in situ remediation protocols are preferred more than ex-situ protocols as they do not involve mobilization of sediments (Lofrano *et al*., 2016). Also, comparing chemical or physical remediation techniques, bioremediation techniques are environmentally friendly, green tech, very sustainable, and cost-effective thereby making them a favored solution to the decontamination of polluted sediments.

With regards to all the current bioremediation technologies, the bioelectrochemical systems (BES) have gained audacious popularity due to their utilization of the powerful abilities of electrogenic microbes to bring about the degradation of petroleum hydrocarbon coupling an electrochemical connection with solid-state electrodes as electron acceptors and donors immense in a hydrocarbon polluted sediment thereby generating electric current through oxidation/reduction (redox) reactions (Aulenta *et al*., 2011; Lovley, 2011, Li & Yu 2015). *Geobacter metallireducens* has been reported with the capacity to oxidize toluene with graphite electrode at +500mV versus the hydrogen electrode with potentiostat which served as electron acceptor (Zhang *et al*., 2010). Morris and Jin (2012) demonstrated that employing a carbon-based anode and cathode exposed to air can greatly improve the biodegradation of hydrocarbon polluted sediment - this method was employed in this research work with a positive outcome. Hence the bioremediation of organic environmental pollutants by way of harvesting the power of electrogenic organisms using BES to catalyze the oxidation of petroleum hydrocarbon in sediments is an emerging technology as it provides the potential for sustainability and continuous degradation, cost-effectiveness, low maintenance, and ultimately a green technology.

In summary, the BES system tries to provide electrogenic microbes (Bacteria & Archaea) domiciled in the sediment with high redox capacity through oxygen as the ultimate electron acceptor in the oxic zone of the unit thereby expediting oxidative biodegradation of the hydrocarbons as contaminants.

The aim of the current study was to construct a lab-scale BES system specifically for the bioremediation of marine sediments contaminated with different types of petroleum hydrocarbons and assess the performance of this system.

2 LITERATURE REVIEW

2.1 Petroleum hydrocarbon description and classification

Petroleum hydrocarbons are a major class of organic compounds consisting of carbon and hydrogen as the hydrocarbon name implies making them one of the simplest compounds on earth. It is important to state here that the classification of hydrocarbons as hydrocarbonaceous compounds containing other compounds other than carbon and hydrogen is incorrect (Speight, 2017). Although with regards the fossil fuel comprising of varying mixtures in combination with some nonhydrocarbon compounds such as nitrogen, oxygen, and sulfur when there is a spill due to anthropogenic activities has made it even more difficult to clearly distinguish hydrocarbons and their derivatives (Speight, 2017). Figure 1 illustrates the classifications of PHC and its derivatives.

2.1.1 Aliphatic hydrocarbons

They are classified by the ratio of carbon to hydrogen atoms therein referred to as being saturated or unsaturated. They are made up of three main groups as a result of the chemical bond present. They are alkanes, alkenes, and alkynes. Alkanes are otherwise known as “Paraffin” and are regarded as saturated with only a single bond while alkenes are known also as “Olefins” and are unsaturated with double bonds. Alkynes on the other hand are made up of triple bonds. Cycloalkanes do not contain multiple bonds while cycloalkenes and cycloalkynes contain double bonds like in olefin or triple bonds as in alkyne (Speight, 2017).

2.1.2 Aromatic hydrocarbons

The term aromatic hydrocarbons are a class of HC that consists of one or more aromatic rings. They were grouped as aromatic as they are structurally obtained from benzene and are more chemically stable than the aliphatic class with benzene as the most stable aromatic compound made up of a single ring with conjugated double bonds. They are unsaturated in their chemical nature. Examples include benzene, toluene, ethylene, and xylene (BTEX). Monocyclic aromatic hydrocarbons contain one benzene ring as part of their chemical structure. In contrast, polycyclic aromatic hydrocarbons (PAH) are compounds that contain more than three aromatics (benzene) rings with notable examples as naphthalene and anthracene (Speight, 2017).

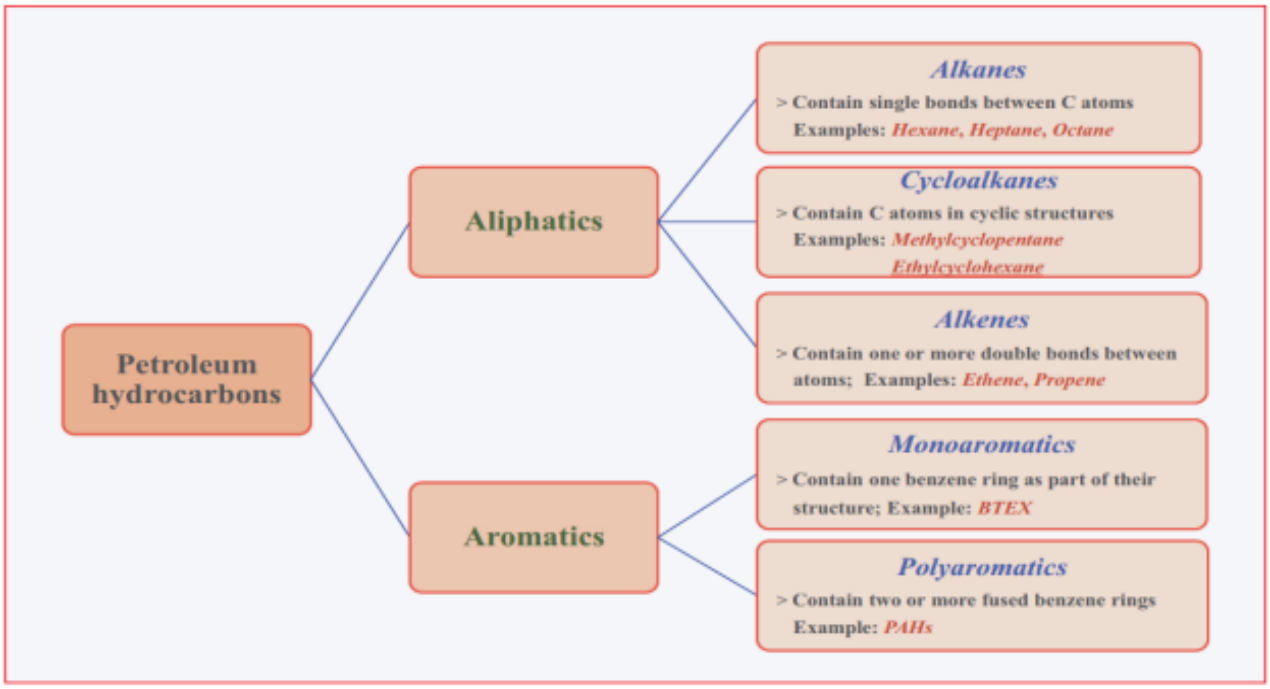

Figure 1. Classification of petroleum hydrocarbons **(**Kuppusamy *et al*., 2020).

2.2 Petroleum hydrocarbon as a natural pollutant - its fate in the marine ecosystem

The inadvertent or intentional release of oil into the natural ecosystem such as air, water, and soil poses a substantial pollution worry. It is therefore important to note that the degree of harm or toxicity varies relative to several factors such as the timeline of exposure or release, the structure of the hydrocarbon, the amount or quantity of spilled substance, and even the channel of spillage which might arise from marine shipping routes, natural seeps, urban and municipal runoff (Kuppusamy *et al*., 2020; Zhou *et al*., 2014). The release of these PH poses a severe ecological threat as they spread in and on surfaces of the polluted platform - a process known as "weathering" which tells the fate of the spilled oil (Figure 2) (Kumar *et al*., 2016).

A couple of factors determine the fate of the spilled oil such as the amount of spilled oil, oi physicochemical characteristics, and the ability of the oil to spill further away to neighboring sites or retain its spilled position (Kuppusamy *et al*., 2020).

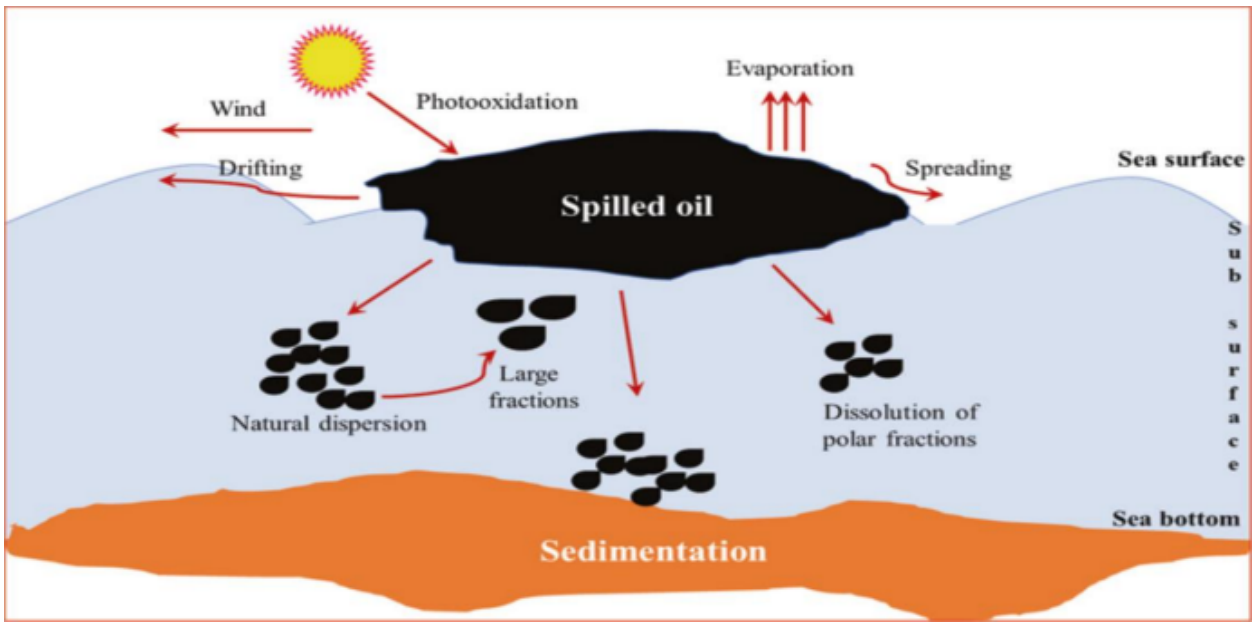

Figure 2. Weathering of spilled oil in the marine ecosystem (Kuppusamy *et al*., 2020).

2.2.1 Arising threat from marine sediments

Polluted marine sediment presents a great challenge in terms of pollutant degradation in the marine ecosystem as a result of all the pollutants from the land, air, and water eventually leaching and accumulating via different geochemical processes into the water bodies and settling as sediments. Examples include natural seeps, surface runoff, adsorption, and precipitation (Li & Yu, 2015). The sediments undoubtedly house almost all difficult to degrade pollutants such as heavy metals, polycyclic biphenyls (PCBs), and polycyclic aromatic hydrocarbons (Sprovieri *et al*., 2007). The role sediments play in marine contamination is extensive as they continue to serve as a sink and a contamination harbor by accumulating contaminants continually over a long period and intermittently releasing these pollutants back into the marine ecosystem thereby serving as a contamination zone (Yan *et al*., 2017).

2.2.2 Statistical overview of petroleum hydrocarbon spillage and effects

One of the greatest burdens on the environment is the crude oil spills. It provides the most adverse impact on the environment. A statistical report spanning over 100 years from 1907 to 2014 estimates that over 7 million tonnes of oil have been spilled, spread over 140 large spills (Etkin & Welch, 1997). In recent times, in the Gulf of Mexico, an estimated 200 million gallons were spilled referred to as the “Deepwater Horizon (DWH)” disaster spanning 84 days of the

release of crude oil in the year 2010, and the death of 11 crew members toppling the 1989 Exxon Valdez disaster of just 5 days. As rampant as these accidents can be, land-based run-off, ship servicing operations, and washing of tanks which we refer to as nonaccidental natural and anthropogenic release of oil accounts for over 90% of crude oil spills leading to an adverse cost-intensive, environmental deterioration, and economic crunch.

Reports indicated that over 5.5 billion dollars were lost to fishing and tourism (Hagerty *et al*., 2010), pollution of marsh shorelines (Lin *et al*., 2012), and thousands of deaths of benthic organisms (Lynne Corn & Copeland, 2011). The Exxon-Valdez spill alone brought the death of over 250 thousand seabirds. The Deepwater Horizon disaster took a whooping cost of 61 billion US dollars.

2.3 Current response techniques to oil spills in the marine environment

The emergency of an oil spill in an environment prompts a standardized and systematic study known as " Environmental Impact Assessment" (EIA) to determine the weightiness of such a spill and to proffer the best-fit response to tackle such a disaster. Emergency response can be mechanically scooping to contain and recover spilled oil, can be chemically by the addition of chemical dispersants or physical cleanup.

Oil spill containments involve different kinds of approaches, for example, mechanical strategy employs booms, skimmers, and barriers including the use of natural or synthetic sorbent materials. The booms are employed as barriers in surface waters to contain and prevent the oil from spreading into other marine bodies. A boom can be curtains, fences, or inflatable booms allowing the aggregation of spilled oil into loops with bigger surface area making it easier for removal by skimming. Accidental release of oil into the sea surface can be contained using plastic booms physically and can be recovered by employing a skimmer strategy or undergoing burning on-site (in situ). Skimmers such as suction skimmers, and oleophilic are available for different soil types. Sorbents are specifically employed for small spills, they are chiefly synthetic-like polymeric materials or natural materials to encourage adsorption of oil spills through swelling. Remnants of oil after skimming are mopped up using sorbents. A mixture of surfactants and solvents that concentrate spilled oil into small particles or droplets usually less than 100μm in size are called dispersants. Dispersants are majorly employed in open deep waters where they promote the dissolution of oil by stimulating microbial mediated oil biodegradation (Kleindienst *et al*., 2015). Hydrocarbon spills far into coastal lines leaking into beaches and can

be reclaimed by sorbents. Strong water spray coupled with sorbents is employed to remove HCs on rocky beaches. Leaking oil wells and pirate activities during oil rigging operations necessitates the leaching of oil into the seafloor and spreading to form HC plumes in the water which in turn spreads all over the sea surface (Kleindienst *et al*., 2015). Dispersants help concentrate the oil into droplets thereby increasing the surface to volume ratio. It is very important to mention that the very first line of action in oil well leak is the wellhead capping to prevent more release of oil into the sea and quickly augmented by other approaches as we have described and will describe in the coming paragraphs. The effect of dispersant application on microbial community composition and metabolic activity is not clear or somewhat controversial. This may be due to differences in applied lab methods, the microbial community involved, or even the type of chemicals used in the production of the dispersant but in all fairness, these dispersants poss some form of toxicity to the microbial communities hence the need for the use of nontoxic biosurfactants in place of chemical surfactants. If a dispersant would produce desirable effects, it depends on several variables such as the temperature of the water body at the time of application, water salinity, and also of great importance is dispersant quantity (dispersant-to-oil ratio), whether the HC is saturated or unsaturated, oil viscosity and oil composition in general (Canevari, 1984; Fingas *et al*., 1989; Fingas *et al*., 1995).

Rising oil contamination has brought about the advancement in methodologies to reclaim spilled oil. In situ burning is a physical method for oil removal, it is a coordinated and controlled burning at the site of pollution to reduce the magnitude of surface oil. This method was employed in the Deepwater Horizon accident where a greater percentage of spilled oil was burned relative to the quantity reclaimed by other means. Another form of HC removal is the natural attenuation and dispersion phenomenon in the water column and volatilization (Kleindienst *et al*., 2015).

In the open sea, an oil spill accidentally occurs from leaking oil tankers en route. These spills can also be from offshore rigging activities on site in the sea or even mishaps like a shipwreck. This spill can be reclaimed by the use of chemical dispersants or physical mechanisms such as skimming and plastic booms or even open in situ burning as seen in the DWH oil spill reclaiming a greater percentage of spilled oil. As the oil leak spreads, it contaminates neighboring beaches. On rocky beaches especially, a combination of oil-sorbent materials and high-pressure water spray can be employed. Sediments can also be excavated for (ex-situ) treatment. Oil penetration through the water column down to the seafloor forms plumes and

spreads to coastal areas. Natural recovery strategies like biostimulation, bioaugmentation, and biosurfactant application enhance the removal of hydrocarbons. (Mapelli *et al*., 2017).

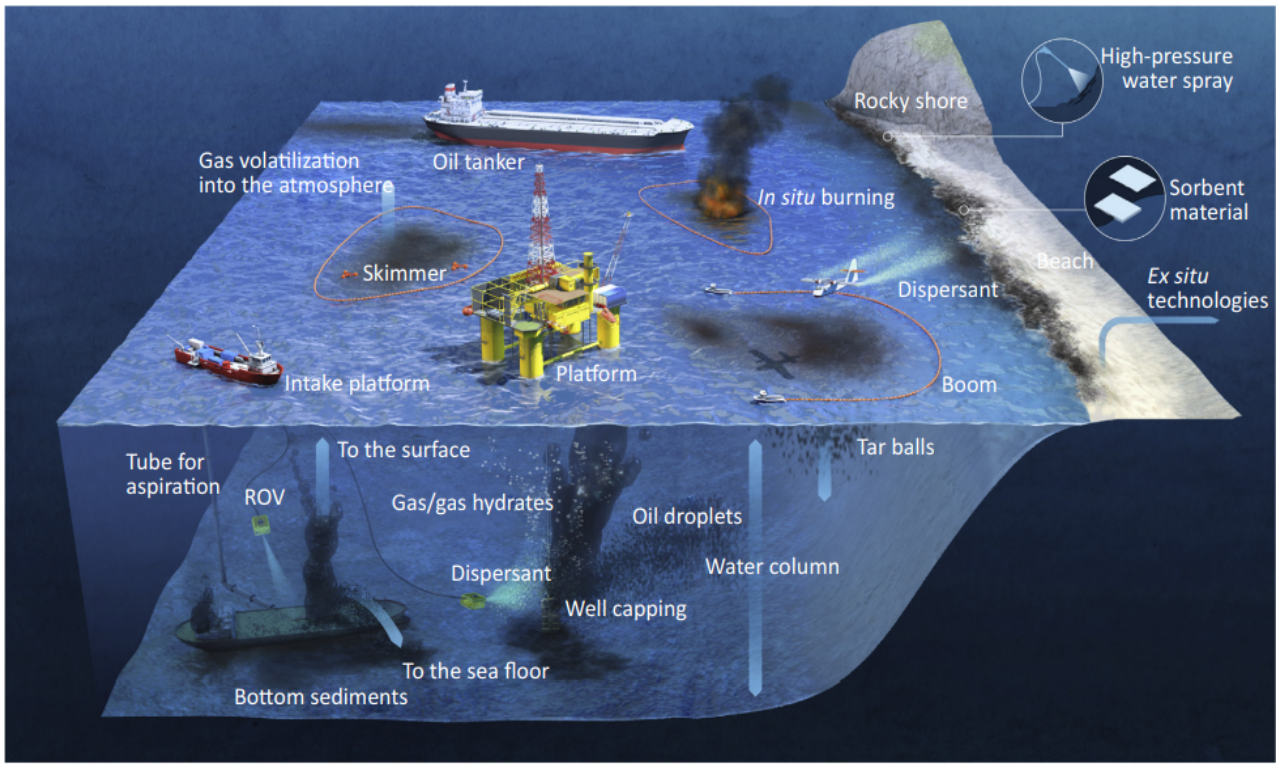

Figure 3. Technologies for oil spill clean-up (Mapelli *et al*., 2017).

2.3.1 Bioremediation and natural attenuation

Bioremediation can not ensue if little or nothing is known about the microbial communities at work in the oil biodegradation process including environmental determinants that necessitate the biotic and abiotic interactions, microbial viability, and their potential to degrade such pollutants. Hence microbes in the marine ecosystem that degrade HC are majorly grouped on a narrow range of HCs or a wider range HCs as either specialists or generalists respectively. Hydrocarbonoclasic bacteria are widely studied because of their biotechnological importance, microbial physiology, and ecological potential giving them more uniqueness and relevance in science. Other strains of bacteria, archaea, and fungi are also noteworthy in the HC-degrading consortium.

Natural attenuation is the aggregation of a complex metabolic network exhibited by an organism relative to other organisms in a micro-environment. These interactions might be between bacteria to bacteria, bacteria to algae or fungi, and vice versa. Low HC solubility poses a threat to HC biodegradation because of high salinity in seawaters. Key player organisms like *Alcanivorax spp*

can produce biosurfactants that promote the availability of HC. A prominent environmental condition that necessitates HC degradation in a particular environment is oxygen availability. Under aerobic conditions, enzymes like alkane hydroxylases encoded by *almA*, *p450,* and *all* genes incorporate oxygen atoms into HC molecules producing alcohols that are oxidized via the **β**-oxidation pathway to yield carboxylic acids (Liu *et al*., 2017; Rojo, 2009). On the other hand, in an anaerobic state, HC degradation is catabolized by alkyl-succinate synthases via the addition of fumarate to the secondary carbon atom. Meta-omics data has aided significantly in the elucidation of complex molecular pathways in the catabolism of HC in situ. The initial response to an oil spill in an environment depends on the type of indigenous or autochthonous microbes present coupled with available environmental conditions. Although 16S rRNA gene sequencing might not capture some specialized HC-degrading bacteria, the metabolic fluxes observed for both aerobic and anaerobic degraders suggest that along polluted marine environment contains much higher microbial diversity as compared to freshly polluted sites (Bargiela *et al*., 2015).

2.3.2 Biological approaches for remediation of water and coastal pollution

Biotechnologies for biological remediation of oil spill sites and the environment should be compatible with the Earth's natural cycle known as the biogeochemical cycle. Bioremediation techniques can be applied in the form of biostimulation, bioaugmentation, or biosurfactant amendments. Biosurfactants are very crucial to the clean up of oil spill sites as they help in the formation of oil in droplets on the water bodies hence making it a lot easier for microbes who feed on HC and are also available or accessible for non-biosurfactant producing microbes. The foremost biosurfactant producers are bacteria, yeast, and fungi and they produce anionic/neutral surfactants. Majorly found in oil spill sites are the *Alcanivorax spp* and some notable *Acinetobacter spp*. Biosurfactants are more advantageous and environmental friendly when compared to chemical dispersants owing to their non-toxic nature, solubilization, ability to form emulsions (oil droplets), high degradation potential, and high stability under extreme pH, temperature, and salinity conditions (Hu *et al*., 2013; Silva-Castro *et al*., 2013). Studies on the impact of chemical dispersants have been addressed in several articles (Kleindienst *et al*., 2015) and have been shown to limit the degrading potentials of biodegrading microbes, while biosurfactants have been used for HC degradation to ease pipeline clogging, oil recovery in reservoirs, and oil emulsification.

The role of inorganic nutrients cannot be over-emphasized as nutrients play a crucial role in bioremediation. Biostimulation is the method or strategy of supplying needed nutrients to indigenous microbial communities to stimulate their natural activities of degradation and aids in the restoration of the nutrient cycle in an unbalanced environment especially after pollution with HC has occurred. Nutrient supply increases the growth of indigenous HC degraders.

Biostimulation of a contaminated site using various sources of nitrogen has been shown to increase HC degrading catabolic pathways without necessarily distorting the structure of the microbial community (Bargiela *et al*., 2015). Apart from nutrient supplementation, microbes that produce biosurfactants also play a vital role in a successful cleanup exercise.

Bioaugmentation is the strategy or method of applying allochthonous non-indigenous microbes to a polluted site to complement the activities of the autochthonous microbiota. HC-degrading microbes cultured under laboratory parameters can be used for bioaugmentation to augment the activities of primary organisms and to improve biodegradation. Often the problem of adaptation of the allochthonous microbe to the site of pollution is encountered, this barrier can be mitigated by employing autochthonous degrading isolates from the primary contamination site, successfully cultivating them under laboratory conditions, and reintroducing them into the site as a bioaugmentation strategy. Biotechnologies for water and coastal pollution no doubt use; biosurfactants, biostimulation, and bioaugmentation in combination or differently to improve/increase the biodegradation of hydrocarbons.

2.4 Biotechnologies for treatment of oil-contaminated marine sediments

Sea bed floor/sediments stand at the receiving end of every oil spill occurring in the marine ecosystem. These spills find a way to penetrate deep down into the bedrocks through the water column. The processes that aggravate this sedimentation can be weathering as discussed, or even the oil adhesion to particulate matter. It has also been observed that the use of chemical dispersants greatly enhances this process as positive evidence of this oil sedimentation through the water column into the seafloor has been studied. About 1.8-14% of oil penetrated deep down the seafloor in the DWH spill (Valentine *et al*., 2014) using hopanes as biomarker tracer and 0.5-9% sedimentation levels as described in the DWH spill studies (Chanton *et al*., 2014)

The penetration level after the oil sedimentation into the seafloor is subject to the site conditions. It may remain settled on the seafloor as a result of existing anoxic conditions which impede the rate of the biodegradation process. It is important to state that several strategies and approaches

to remediate HC polluted marine sediment especially to encourage or aggravate the number of autochthonous degraders have been proposed at one point or the other but in situ remediation employing bioaugmentation, biostimulation and use of biosurfactants has proved an effective and efficient strategy for such remediation due to its sustainable nature. To bring about the population growth of autochthonous degraders, the addition of biosurfactants, electron acceptors, and nutrients is crucial. The effectiveness of sediment remediation is very dependent on the availability of electron acceptors such as nitrate, iron, magnesium, and sulfate as they tend to increase degradation levels

As an option to enhance oil biodegradation rate in sediments, oxygen can be specifically delivered into the sediment column. An example of this is the modular slurry system (MSS) which applies aeration to increase the removal of polycyclic aromatic hydrocarbon (PAHs) contaminants by up to 98% (Genovese *et al*., 2014). The limitation of this strategy is that it is laborious and energy-intensive. Another strategy is the application of oxygen-releasing compounds such as calcium peroxide-based chemicals which have also proved efficient with its limitations being the problem of controlling the amount of supplied oxygen and the subsequent reaction between oxygen and reduced chemical species such as iron and sulfate.

Amongst the strategies and methods employed in various studies over the years, the most recent and current strategy is the bioelectrochemical system which has been used to increase the biodegradation of HC in marine sediments. As an example, the " oil snorkel" is an innovative bioelectrochemical approach to accelerate HC bioremediation in marine sediments (Cruz Viggi *et al*., 2015). The system is made up of a conducting graphite rod connecting two redox zones namely; the oxic and anoxic zones. The anode is the portion embedded in the anoxic sediment serving as an electron acceptor, this carries electrons produced by the catalysis of HC by electrogenic bacteria in an anaerobic complex and that of biochemical oxidation of reduced species (sulfate and iron). These electrons are carried to the oxic region (the cathode) by redox potential difference which they react with oxygen to produce water as a waste product. This technique stimulates HC degradation and has been employed in several studies. The technique requires no energy input and low maintenance and can be used for a long period in the clean-up of oil spills. This technology has been piloted on a lab-scale and is scaled up for use in the open sea in the foreseeable future.

2.4.1 Hydrocarbon degradation - Actors behind the scene

Carbon source is an intrinsic requirement of microbes and about 175 genera of prokaryotes in an upward of 7 phyla use HC for this essential survival strategy (Figure 4) (Hazen *et al*., 2016). The advance in sequencing techniques has allowed us to identify and systematically categorize members of these species. This genome sequencing technology has made it even more possible the characterization of HC-degrading microbes without necessarily cultivating them in culture conditions. In the past two decades, our knowledge has expanded in the ecological diversity of marine microbes. Some of the notable examples include *Alcanivorax* spp, *Fundibacter* spp, and *Oleispira* spp. Both in aerobic and anaerobic conditions in different marine ecosystems, different microbial species exist within the niche because of one or two favoring conditions. HC-degrading microbes that tolerate oxygen availability has been identified both in the water column where there is a constant penetration of spilled oil during such a disaster and deep down in the oxic sediments of the open seas while anaerobic HC-degraders are predominantly identified in anoxic sediments. Notable studies in the Arctic (Mcfarlin *et al*., 2014), Antarctic (Delille *et al*., 1998), and polar ice region (Barker *et al.*, 2011) have proved the biodegradation of oil even under extreme environmental conditions.

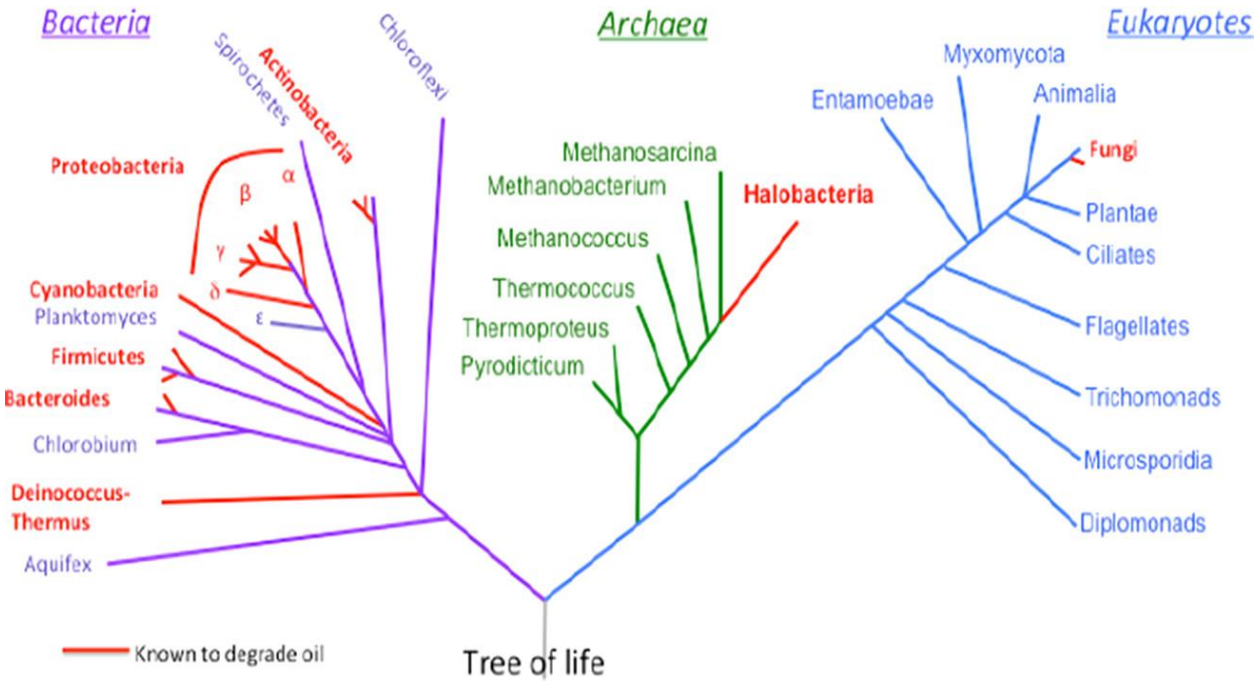

Figure 4. Oil degrading microbial taxa. Oil degrading microbial phyla, highlighted in red, have been identified from all three domains of life (Hazen *et al*., 2016).

2.5 Bioelectrochemical system for organic pollution removal

BES has been defined as the technique that employs/harvests the potentials of electrogenic microbes to fully catalyze the oxidation/reduction reactions with the aid of organic matter with the help of a conducting material known as an electrode (Rabaey *et al*., 2009). In a typical BES unit, the is a compulsory association between the microbe and the conducting material. This association can be either direct or indirect. Direct association entails when the electrogenic microbe directly shares electrons with an electrode or indirectly when a mediator compound acts as an electron carrier. These electron carriers can be metabolites produced by the microbe such as phenazines of *Pseudomonas spp* (Rabaey *et al*., 2005) or these mediator compounds can be artificially incorporated into the unit (Logan *et al*., 2006). Electricity generation is an output characteristic of the BES unit in MFC setup by the integration of the sediment oxidation in the anode zone and the reduction at the cathodic zone coupling oxygen as the ultimate electron acceptor (Logan *et al*., 2006).

2.5.1 BES classification/type

The BES has been invented over the years and has constantly been improved to bring efficient and total catalysis of organic contaminants using electrogenic organisms (Rabaey *et al*., 2009). The unit consists of the anode and cathode zones with a conductive material bridging the gap (Daghio *et al*., 2017). This study employs MFC for the degradation of PHC.

2.5.1.1 Microbial Fuel Cell

This system involves the degradation of contaminants in organic matter like sediment and soil by the activities of autochthonous microbes leading to a spontaneous generation of electric current as a positive result of active biodegradation.

By harnessing the powers of electrogenic microbes to oxidize sediments at the anode connected to a resistor through the cathode, electricity is produced which is an indication of the organic matter biodegradation (Srinivasa Raghavan *et al*., 2017). Due to the advanced state of the MFC, it has been applied on a grand scale in various fields of research such as in the production of renewable energy by wastewater treatment (Patil *et al*., 2009), decomposition of hydrocarbons including methane production, agricultural and urban waste treatment (Yi *et al*., 2009; Evelyn *et al*., 2013).

2.5.1.2 Working principle of BES

The mode of operation of the MFC is primarily by the activities of electrogenic microbes who bring about the oxidation of substrates (sediments) by a catabolic process ultimately generating an electric current. In the case of acetate as the final substrate from the catalysis of organics in the unit, the reaction that plays out in the anodic zone and cathodic zone are thus illustrated (Adelaja *et al*., 2015).

Anodic chamber: $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$ ……(1)

Cathodic chamber: $O_2 + 4e^- + 4H+ \rightarrow 2H_2O$ ……..……...…(2)

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

Firstly, in the anode zone of the unit, microbes bring about decomposition of the sediment producing hydrogen ions and electrons. The electrons are transported as earlier mentioned by direct or indirect mediation (see BES system) from the anodic zone (site of electron production by the catalysis of the contaminated sediment) to the cathode. At the same time, protons (hydrogen atoms) are transported by the proton exchange membrane/cation exchange membrane (PEM/CEM) from the anodic zone to the cathodic zone. Electricity is produced on the load blinker board circuit as flashes of light.

Secondly, in the cathodic zone, oxygen reduction occurs forming water. Further acceptance of the electrons and protons by oxygen at the cathode initiates further diffusion of protons and electrons from the anode zone by the microbial substrate (sediment) catalysis (Logan, 2009; Du *et al*., 2007, Kumar *et al*., 2016).

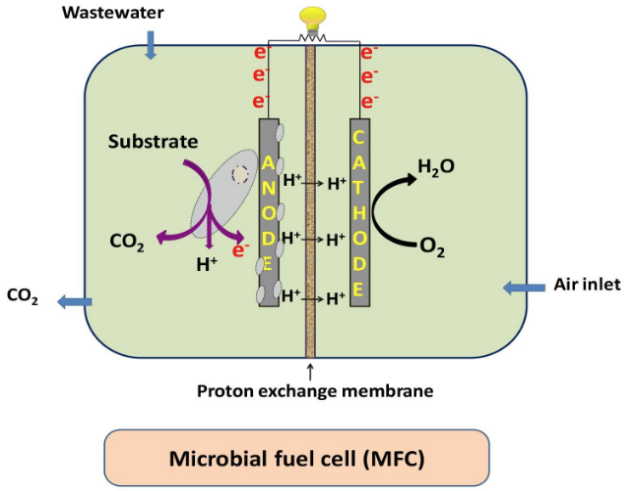

Figure 5. Microbial fuel cells with configurations and mechanisms (Shanthi Sravan *et al*., 2021).

2.5.2 Application BES for oil spill remediation

2.5.2.1 Oxygen production and anode oxidation

Before the present use and application of the BES, it was invented primarily for power generation through the reduction of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in wastewater treatment plants (Logan *et al*., 2006). Presently, its applications have expanded to the oxidation of PHC for bioremediation processes. The anode which is immersed in the contaminated sediment in an anaerobic condition picks up released electrons due to the catalysis of the organic pollutant. Electrons released at the anodic zone flow through a conductive material to the cathode where they reduce oxygen in anoxic conditions (Lovley & Nevin, 2011). Petroleum hydrocarbons and their derivatives; aliphatic and aromatic hydrocarbons are degraded using BES. Among the BTEX, toluene is readily degradable and has been extensively experimented with different electrode potentials with autochthonous and allochthonous cultures (Zhang *et al*., 2010; Friman *et al*., 2012, Lin *et al*., 2014).

Zhang and co-workers (2010) demonstrated the degradation of benzene at the anode using allochthonous enrichment cultures. Groundwater and wastewater were demonstrated by Rakoczy *et al*. (2013) and Wu *et al*. (2018), respectively. Anaerobic sludge degradation was demonstrated by Adelaja *et al*. (2015). Studies on phenol degradation both in pure and mixed cultures of *Cupriavidus basilensis* (Huang *et al*., 2011). It is therefore important to note that at in situ contaminated sites, hydrocarbons are present in mixed fractions known as total petroleum hydrocarbon (TPH) which has been studied to a large extent (Morris & Jin 2012; Cruz Viggi *et al*., 2015; Li *et al*., 2015).

2.5.3 Effect of BES operational parameters and materials

2.5.3.1 Electrode materials

The type and choice of conducting materials can either improve or retard the biodegradation process and hamper the efficiency of the decontamination process. Therefore, according to Daghio *et al*. (2017), good conducting material should:

1) Possess high electrical conductance;
2) Possess chemical reactivity and physical stability;
3) Possess high catalytic potentials;

4) Should be sensitive and selective to target compounds;
5) Should be relatively inexpensive.

For the most complete biodegradation to occur, the material preference is key as the type of material detects the form of oxidation that would take place competing with oxygen evolution potentials (Anglada *et al*., 2009). If the electrode material surface is not compatible with the electrogenic microbes, they scarcely attach to it or reduce their overall adhesion to such a surface. Standard electrodes commonly used in BES include carbon electrodes made up of carbon matter, rod, mesh, and metallic steel; both types are compatible with degrading microbes and do not corrode (Ghangrekar & Neethu, 2020).

2.5.3.2 Carbon materials

Carbon materials have been experimented with in numerous BES units due to their large specific surface area (SSA), inert chemical state, cost-effectiveness, and super conductance. However, a notable disadvantage of carbon materials is the clogging of the carbon pores by the formation of microbial biofilm which decreases their electrochemical reactivity (Dumitru & Scott, 2016). Yet another key deciding factor is the surface of the carbon material as this will greatly determine if microbes will attach to the anode and the subsequent transport of electrons to the cathode. These drawbacks can be mitigated by applying various surface modifying methods that guarantee the success of the electrochemical process.

2.5.3.3 Improved conducting materials - metals

The use of metals as conducting materials has been studied with great positivity when used as anodes in BES. Among these notable metals are stainless steel and titanium which have been implicated in several studies. (Dumitru & Scott, 2016) alluded to the ineffectiveness and poor conductance of copper electrode materials due to their corrosiveness and toxicity to several microbial species even as a more stable element compared to carbon. On the other hand, the mechanical characteristics of steel including its non-corrosiveness have made stainless steel a wise option and have been demonstrated in anode and cathode compartments in BES with long-term durability (Papillon *et al*., 2021). Erable and Bergel (2009) alluded to the positive outcome when using stainless steel under standard inoculation with microbes. Studies reported on the use of titanium in combination with graphite-coated titanium showed great conductance, unlike the uncoated titanium which could not transport electric current (ter Heijne *et al*., 2008).

Researchers have also demonstrated the effectiveness of Gold as a conducting material with high conductance and microbial attachment properties but cannot be sustainable as Gold is unimaginably cost-intensive hence, not a good choice because of the cost involved (Choi & Chae, 2013; Qian *et al*., 2009).

2.5.4 Merits and demerits of BES

The advantages of BES are enormous. One very important advantage is the ability to convert harmful pollutants into an innocuous state by manipulating the anode/cathode potentials coupled with the fact that electron flow could be maintained for a long period. Since the process is spontaneous, there is no need to add exogenous chemicals thereby reducing the cost of transporting and securing these chemicals. BES is a green technology and poses no threat whatsoever to the environment. The materials are also not expensive. Another merit is that the chemical techniques produce a high amount of toxic waste from the degradation of specific pollutants while BES does not produce any toxic substance since they have wider and broad selectivity over the chemical techniques. (Wang *et al*., 2011) demonstrated in their study the biocatalysis of nitrobenzene to aniline produced less toxicity with 99% degradation potential, while in another study using chemical methods, nitrosobenzene was produced which is a more toxic compound and highly recalcitrant (Mu *et al*., 2014).

BES is not without some limitations, the highest limitation of this technology is the slow process of degradation. The rate of oxidative degradation at the anode is yet another factor to consider as a drawback including the cathode quality (Liu & Logan, 2004). Another factor to be wary of is the production of chlorine gas especially in the marine ecosystems since this gas reacts readily with both organic and inorganic matter forming harmful and toxic substances (Richardson *et al*., 2007). Therefore for the best BES, care should be taken to select compatible materials, and possibilities for future scale-up to industrial operational capacity should be considered.

2.5.5 BES Applications

BES use and applications gained much popularity solely for their ability to remediate organic and inorganic pollutants. Let's now examine briefly some of the areas of application.

2.5.5.1 Biodegradation of petroleum hydrocarbon

BES has been applied to the bioremediation of hydrocarbon-contaminated marine sediments. (Cruz Viggi *et al*., 2015) demonstrated the application of BES in HC degradation using the bioelectrochemical snorkel with rod graphite as an electrode immersed in fuel-oil contaminated sediment with a cathode zone in the oxic overlying water. After 200 days the result shows a 21% reduction in TPH with a similar increase in reduction of TPH after 417 days. In another study using river sediment contaminated with crude oil, MEC brought about the degradation of TPH and improved sulfate reduction (Viggi *et al*., 2017). *Proteobacteria* was the prominent phylum in MEC remediation of marine sediments. The MEC conducting material surface had predominantly *Alphaproteobacteria* which includes sulfur-oxidizing bacteria transferring electrons from sulfide oxidation of the sulfur-containing sediments (Matturro *et al*., 2017).

2.5.5.2 Wastewater treatment

BES has been extensively applied in the treatment of wastewater such as the study on the MES COD reduction potentials which gave a 75% removal rate compared to the MFC COD reduction of 50% (Erable *et al.*, 2011). Similarly, a study by (Ramirez-Vargas *et al*., 2019) demonstrated that MES application in wetlands gave assuring positive results. When used as an electroconductive biofilter applied in a man-made lagoon compared to the natural lagoon with fine stones and gravel, the result showed 3-4.5 degrees of time saved with regards to BODs and CODs removal process (Aguirre-Sierra *et al*., 2016). Another study by (Ramirez-Vargas *et al*., 2019) demonstrated the application of MES in the treatment of pig manure relative to the biosand filter technique, MES attained a 90% COD removal and 81% using the biosand filter.

2.5.5.3 Nitrate removal

BES has been studied in denitrification processes. In a study by a researcher (Yang *et al*., 2015), a case study was demonstrated where carbon felt was fused into contaminated sediment acting as an anode, and a corresponding iron cylinder immersed in a $NaNO_3$ solution as a cathode. The result of this set-up showed a massive reduction of NO_3 to NO_2 at the cathode by the oxidation of sediments at the anode. After 16 days of the experiment run, a 98% denitrification effect was achieved (Yang *et al*., 2015).

2.5.5.4 Soil bioremediation

The state of soil determines the extent to which bioremediation can be achieved. For instance, a waterlogged soil referred to as “Flooded Soil” has fewer electron acceptors such as O_2 and NO, and this, in turn, limits the microbial-assisted removal process. This condition is primitively amended by artificially adding such electron acceptors which produce some toxic end product. A modern technique is the “bielectronventing” method which is comparable to the BES such as in MES for the decontamination of soil polluted with the herbicide atrazine to CO_2 which is easily degradable (Dominguez-Garay *et al*., 2017). In the MES study for soil bioremediation, graphite felt electrodes were used, immersed into the soil vertically and unto the overlying water. After 20 days of experiment monitoring, there was an 80% detoxification of atrazine in the MES compared to the 55% in the control unit (Dominguez-Garay *et al*., 2017).

2.5.5.5 Metal recovery

Among the studies of BES on metal recovery is that of (Mitov *et al*., 2021) who demonstrated that copper can be recovered from an aqueous solution. Two MES were used, one with membrane and one without membrane compared to the MFC system. In 2 days, MES without membrane achieved a 95% copper recovery from a solution of aqueous metals while MES with membrane and the MFC unit had a 97.8 ± 4.5% and 98.3 ± 4.8% recovery in 10 days respectively (Mitov *et al*., 2021). This study and similar others alike have demonstrated the efficiency of BES in metal recovery.

3 THE AIMS OF THE THESIS

- To construct a bioelectrochemical system powered by the activities of electrogenic microbes to degrade and remediate oil pollution in marine sediments from the oil shipping Baltic Sea route.
- To measure the degradation rate of oil and oil products in the sediment in BES units.
- To characterize the microbial community structure and abundance in BES units using quantitative PCR and amplicon sequencing.

4 EXPERIMENTAL PART

4.1 Materials

Amongst the aims of this thesis project was the construction of a BES to initiate the biodegradation of PH bringing about the bioremediation of the pollutants therein through the activities of electrogenic microbes. To this end, we began by sourcing materials necessary for the project. Sediments are our prime material for this project. Sediment samples were obtained from the entrance of the Gulf of Finland (59.581850, 23.62683) at a depth of 80 meters.

The electrodes we used consisted of anode felt and wire plus cathode felt and wire made up of carbon graphite materials. Contaminants were used to artificially spike the sediments were crude oil (CO) - a raw/crude mixture comprising hydrocarbons; intermediate fuel oil (IFO) - a high capacity machine oil used primarily as vessel fuel worldwide for ocean liners; dispersant (Finasol 52) - a chemical dispersant added to aid the emulsification of oil into droplets for easier degradation. We used and tested a set of resistors in the range of 47, 120, 200, 470, 1k, 2.2k, and 4.7k ohms for the capacitance of which we later settled for 470k ohms resistor due to its stable capacitance in measuring the potential difference across the electrodes during current generation. We used alligator and jumper wires for connections with the multimeter. Our BES was run in real-time, monitoring the level of degradation which is the objective of the BES construction. To this end, we used a Picolog ADC-24 Multichannel Data Logger (Pico Technology, UK) which measures real-time voltage generation as the electrogenic organisms contained in the unit carry out biodegradation and thereby release electrons transmitted as electric current (see BES working principle). Finally, to measure current and resistance we used the RS PRO S3 handheld digital multimeter. The aforementioned materials were put together in the construction and set-up of three bioelectrochemical system units.

4.2 Experimental Set-up of BES/Construction and Operation

This experiment was carried out using marine sediments from the Baltic Sea. The sediments were all evenly mixed together to allow and encourage the homogenous distribution of sediment properties. The sediment was spiked in the laboratory with crude oil (CO), Intermediate Fuel Oil (IFO), and Dispersant (D); “Finasol 52”. The three BES units were connected in a parallel circuit

connection to the Picolog logger that monitors and extracts readable voltage data units simultaneously.

Each of the three units contained 300 g of sediment and 15 g of contaminant, CO, IFO, and CO+D, respectively. The ratio of mixing was 1:20 except for the CO+D unit in the ratio of 300:15:1. Where 1 represents 1g of dispersant (Finasol 52). In addition to BES units, three control units without electrodes were set up in the same ratio.

The conductive graphite fiber anode and cathode wires were bent to a 90-degree where the plastic sheath ends, then the bare end of the graphite fiber wire is straightened. The bare end of the anode wire was inserted into the side of the thin felt disc straight up without the wire poking out of the felt. This was repeated for the cathode wire. A layer of the contaminated sediment with a thickness of 1 cm was packed deep into the bottom of the BES unit vessels making a leveled smooth layer underneath the vessels. The anode graphite fiber was placed on top of the 1 cm smooth leveled sediment in each of the three units pressing down firmly to squeeze out all air bubbles. The units were filled up with more sediment from the respective mix pressing down gently to get rid of air bubbles discarding spill-over liquid. The cathode graphite fiber was gently placed on top of the 5 cm layered sediment in each unit leaving the top of the cathode graphite fiber exposed to air. To detect the generation of current, a resistor of 470 Ω was attached to the circuit board onto the cover of each unit poking out the anode and cathode felt wires. The BES units were connected to the picolog ADC-24 multichannel data logger using jumper and alligator wires. The voltage level was monitored in real-time using the Picolog cloud software. The entire experiment after set-up was run for 34 days with corresponding controls.

100g of each sample unit (CO, IFO, CO+D) was collected for chemical (TPH) analysis and 50g of each of the sample units were collected for microbiological analysis. For control analysis, the same measurements were taken approximately for both chemical and microbial analysis.

4.3 Analytical Methods

4.3.1 Electrical Measurements

Power generation is an essential parameter to estimate the performance of a BES unit. In this case, power was calculated using Ohm's law:

$$P = \frac{V^2}{R}$$

where P is the power of the BES unit, calculated in μW, V is the voltage, measured in mV, and R is the resistance used. Polarization data were obtained by changing the external resistance (varied from 120 Ω to 2.2 kΩ) by means of a variable resistor box during the stable power production stage of the experiment.

4.3.2 Total Petroleum Hydrocarbon Quantification

Total petroleum hydrocarbon (TPH) content (Nonpolar TPH C10-C21 and C21-C40, sum of C10-C40) in sediment samples was measured using ISO method 16703:2011 by Eurofins Environment Testing Estonia OÜ.

TPH removal (%) was calculated as follows:

$$\left(\frac{conc\ Day1 - conc\ Day2}{conc\ Day1}\right) \times 100$$

4.3.3 DNA Extraction

DNA was extracted from the sediment samples using DNeasy PowerSoil Pro Kit (Qiagen, Foster City, CA, USA). The manufacturer's protocol was modified by replacing 10 min of vortex treatment with the homogenization of the samples using TissueLyser II (Qiagen, Foster City, CA, USA) at 25 Hz for 10 min. The quantity of the DNA extracts was assessed by Invitrogen Qubit 4 Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The extracted DNA was stored at −20 °C until further analysis.

4.3.4 Quantitative Polymerase Chain Reaction Conditions

Quantitative Polymerase Chain Reaction (qPCR) was used to determine the abundances of 16S rRNA genes specific to bacteria (B16S) and archaea (A16S). The qPCR assays were performed on RotorGene® Q with RotorGene Series Software v 2.0.2 (Qiagen, Foster City, CA, USA). The qPCR reactions were performed in a 10μl volume containing 5 μl of Maxima SYBR Green Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), an optimized concentration of forward and reverse primers, 1 μl of template DNA, and sterile distilled water. qPCR details are given in (Table 1). Immediately after the qPCR assay, a melting curve analysis was performed by increasing the temperature from 70 °C to 90 °C (0.35 °C/3 s) with continuous fluorescence recording. All qPCR samples were measured in triplicate, and negative controls were included in every qPCR run. Amplification inhibition was assessed by comparing amplifications of dilution

series from the targeted samples and twenty-fold dilutions of samples were used for amplification to negate inhibition.

Quantification data were analyzed with the LinRegPCR program v 2021.2 (Rujiter *et al*., 2009). The target gene abundance was calculated through the estimation of the fold difference between a sample and multiple data points from the standard curve, as described by Nõlvak *et al*. (2016), and is presented as gene copy numbers per gram of dry sediment weight (copies/g dw). All the targeted ARGs were normalized against the total 16S rRNA (B16S + A16S) gene abundance to represent the relative abundances of ARGs in the prokaryotic community. Additionally, the relative abundance of archaea (%) in the prokaryotic community was calculated.

Table 1. Characteristics of the qPCR and PCR primer pairs and programs used.

Target gene	Primers	Primer sequence 5´-3´	Amplicon size (bp)	Primer concentration (µM)	Amplification program	Primer reference
Bacterial 16S rRNA (qPCR)	Bact517F	GCCAGCAGCCGCGGTAA	530	0.6	95°C 10 min; 35 cycles: 95°C 30 s; 60°C 45 s; 72°C 45 s	Liu *et al*., 2007
	Bact1028R	CGACARCCATGCASCACCT*				Dethlefsen *et al*., 2008
Archaeal 16S rRNA (qPCR)	Arc519F	CAGYCGCCRCGGTAA*	393	0.6	95°C 10 min; 35 cycles: 95°C 15 s; 56°C 30 s; 72°C 30 s	Espenberg *et al*., 2016
	Arch910R	GCYCCCCCGCCWATTC*				
Bacterial 16S rRNA (PCR)	27F	AGAGTTTGATCCTGGCTCAG	1500	10	95°C 2 min; 30 cycles: 95 °C 1 min, 60 °C 1 min; 68 °C 3 min	Heuer *et al*., 1997
	1492R	GGTTACCTTGTTACGACTT				

*- R is G/A, S is G/C, Y is C/T and W is A/T.

4.3.5 Oxford Nanopore Sequencing

The bacterial community composition was assessed using the 16S Barcoding Kit provided by Oxford Nanopore Technologies (UK). The 16S Barcoding Kit offers a method of amplifying and barcoding the ~1500 bp 16S rRNA gene from multiple samples and sequencing them together. The sample DNA is amplified by PCR using specific 16S primers (27F and 1492R) that contain barcodes and 5' tags which facilitate the ligase-free attachment of Rapid Sequencing Adapters. The PCR reactions were performed in a 50 µl volume containing 25 µl Phusion High-Fidelity

polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1 µl of 16S Barcodes, 14 µl of nuclease-free water, and 10 µl of input DNA (10 ng). The PCR reaction conditions are given in Table 1. PCR products were cleaned using Nucleospin Gel and PCR Clean-up kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the amount of recovered DNA was quantified using Invitrogen Qubit 4 Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). All the barcoded libraries were pooled together and added 1 µl of rapid sequencing adapters supplied in the kit. The amplicon library (11 µl) was diluted with sequencing buffer (35 µl) containing 3.5 µl of nuclease-free water and 25.5 µl of loading beads and run on an R9.4.1 flow cell (Oxford Nanopore Technologies, UK) after performing platform quality control analysis using MinION Mk1C device (Oxford Nanopore Technologies, UK). Obtained sequences were analyzed using Kraken2 software (Lu & Salzberg, 2020).

5 RESULTS

5.1 Voltage and power generation in BES units

The electrical characteristics, such as voltage and power generation were measured for a total period of 34 days in BES units (Figure 6; Supplementary Figure S1). Based on the obtained data, the CO+D unit had the most rapid initial increase in the power output. Also, this BES unit had nearly twice higher power production compared to the crude oil BES unit for two weeks period. BES unit with IFO showed the lowest power production but this was rather stable at the time. Both in the case of CO and CO+D units, the power generation dropped after the 25th day.

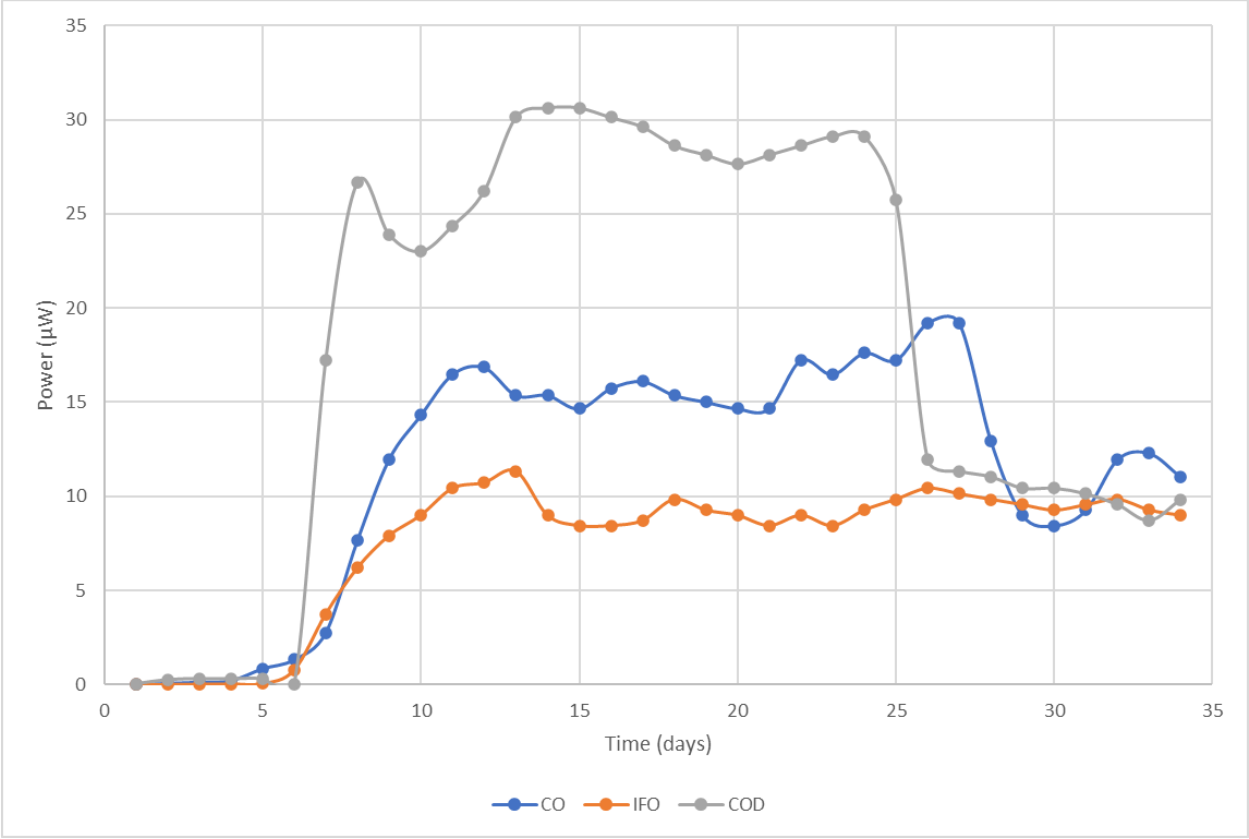

Figure 6. Power generation dynamics in BES units during the experiment.

A closer peep into the power generation potentials in the three BES units, we observed that power generation was highest in unit 3 (CO+D) with 30.7 µW on day 15 although the peak of power generation started on day 8 with 26.7µW and suddenly dropped on day 9-12 after which came to a climb in power generation from day 13-15. These days of high turnover of electric current generation aptly represent the state and level of removal efficiency as the electrogenic

microorganisms were in their optimal operating conditions. According to the microbial growth curve theorem, days, 0-5 across all 3 units represent the "Lag Phase" of the microbial growth where they try to adapt to the medium, at this stage little or no metabolic process has taken place. Day 6-15 represents the "Log Phase or Exponential growth Phase" when the microbial community is actively dividing and consuming the substrate in this case the HC as contaminants in each unit. Day 16-27 shows a "Stationary Phase" of growth where neither further growth occurs but a state of balanced physiology of the microbial communities. Day 28-34 depicts the "Death Phase" a state of gradual decline in power generation and metabolic activities are dwindling and the death of active microbial communities is imminent.

5.1.1 Effect of external resistance on BES

At the end of the experiment, variable external resistances were applied to evaluate the impact of external resistance on the behavior of the BES units. As shown in Figure 7a, the

output voltage of the two BES units (CO and IFO) increased similarly with the increase in the external resistance. But the BES unit with dispersant showed a more rapid rise in voltage up to 1000 Ω. Although an increment of external resistance led to raised output voltage in the two BES units (CO and IFO), the power density was capped in these units at 500 Ω (Figure 7b). Contrary to this, the BES unit with dispersant showed maximum power output when external resistance was 1 kΩ.

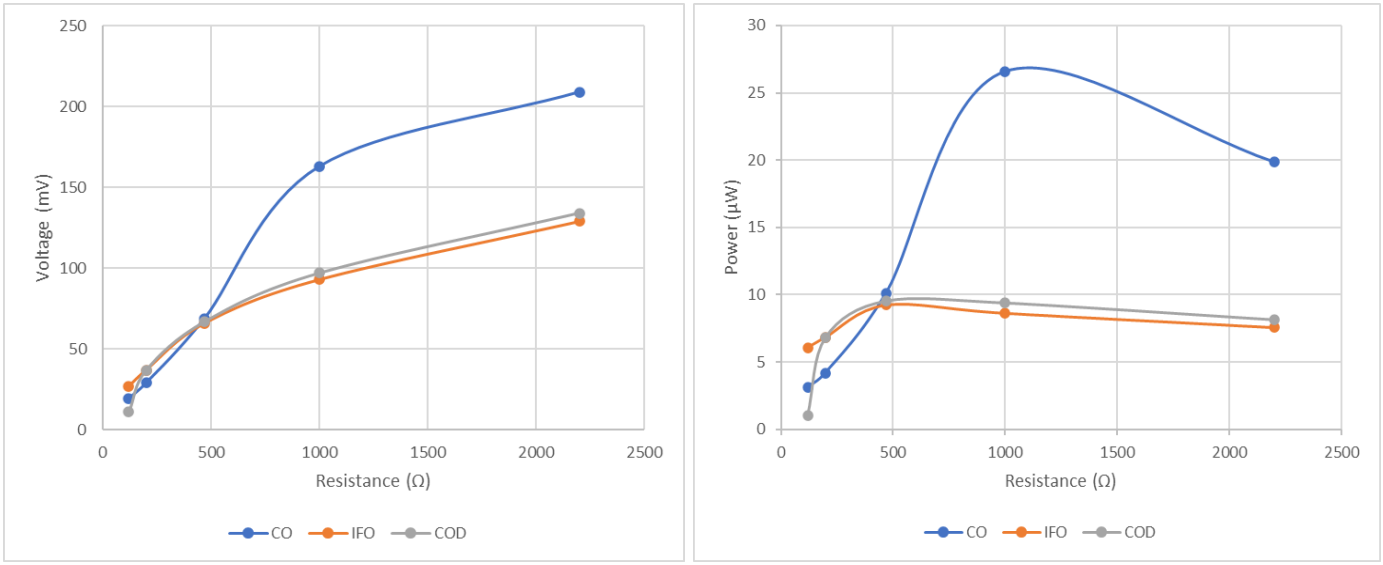

Figure 7. Effect of external resistance on (a) output voltage and (b) power output on 34 days of the experiment.

5.2 Total Petroleum Hydrocarbon removal efficiency in BES units

Table 2. Total petroleum hydrocarbon (TPH) concentration values and removal efficiency at the beginning and end of the experiment in BES units. Shown are concentration values for alkane lengths C10-C20, C21-C40 and C10-C40, respectively.

BES unit	**TPH C10-C21 (mg/kg dw)**			**TPH C21-C40 (mg/kg dw)**			**TPH C10-C40 (mg/kg dw)**		
	Day 1	Day 34	Removal (%)	Day 1	Day 34	Removal (%)	Day 1	Day 34	Removal (%)
CO	77000	57000	26.0	62000	46000	25.8	140000	100000	28.6
IFO	52000	35000	32.6	33000	22000	33.3	85000	58000	31.8
CO+D	77000	43000	44.2	62000	34000	45.2	14000	77000	45.0

The three units of the BES were analysed from day 1 to day 34 for removal efficiency of TPH with alkane length C10-C20, C21-C40 and C10-C40 respectively. Table 2 shows the concentration and removal efficiency values for alkane with chain lengths C10-C21, C21-C40, and C10-C40. The highest oil removal efficiency was achieved in the BES unit with dispersant, while BES units with CO and IFO showed lower removal efficiencies.

5.3 Microbial community abundance and structure in BES units

The abundance of the total 16S rRNA genes in the initial sediment was $1.4x10^{10}$ copies/g dw and ranged from $6.7x10^{9}$ to $2.4x10^{10}$ copies/g dw in the BES units (Figure 8, Supplementary Table S1). The abundance of B16S and A16S slightly increased in the BES units with CO and IFO. In the BES unit with dispersant addition, the abundance of B16S decreased compared to Day 0 while the abundance of A16S dropped two orders of magnitude. The relative abundance of archaea (arc%) in the sediment prokaryotic community was 9% and slightly increased in the CO and IFO BES units but substantially decreased to only 1% in the CO+D BES unit (Figure 8, Supplementary Table S1).

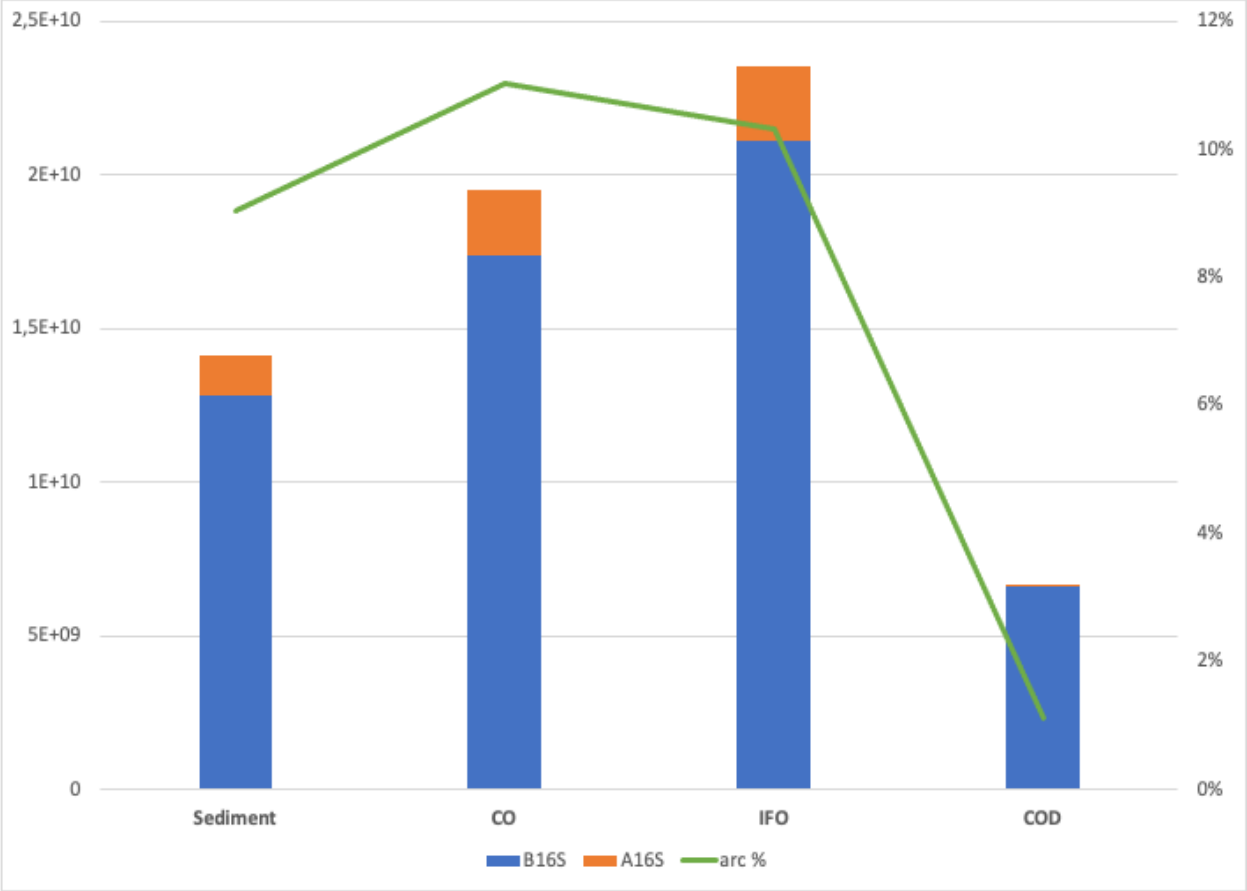

Figure 8. The abundance of bacterial (B16S) and archaeal (A16S) 16S rRNA genes in the sediment and BES units according to quantitative PCR results. The relative abundance of archaea in the prokaryotic community is shown with the green line (arc%). The sediment sample indicates the microbial abundance on Day 0 before spiking material with crude oil and IFO.

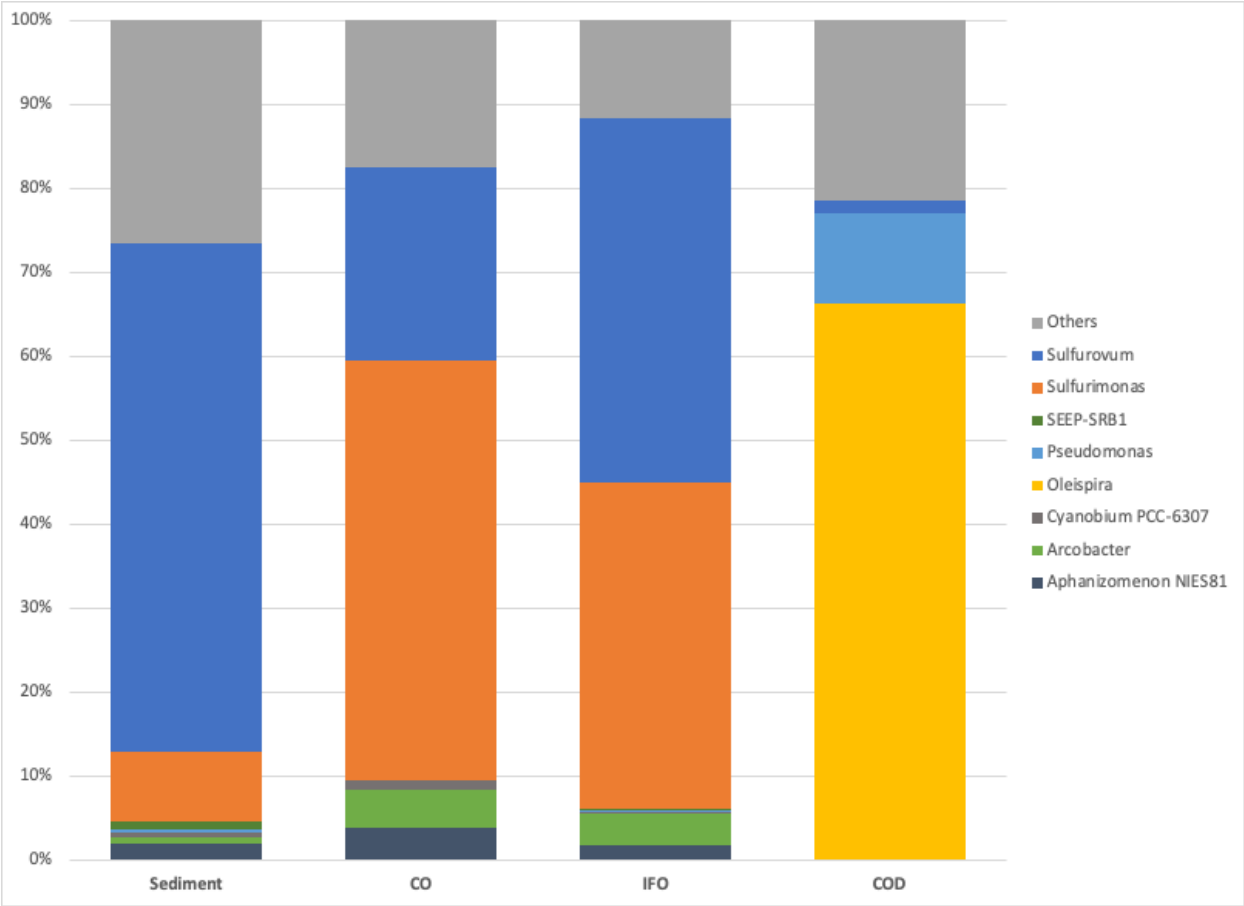

Figure 9. Bacterial community composition on genus level in initial sediment and BES units. Shown are genera with relative abundance greater than 1% in the community.

The bacterial community in the initial sediment sample was dominated by two genera - *Sulfurovum* and *Sulfurimonas* (Figure 9). Genus *Sulfurovum* consisted mainly of two species *Sulfurovum lithotrophicum* and *Sulfurovum aggregans*. Genus *Sulfurimonas* consisted of two species- *Sulfurimonas gotlandica* and *Sulfurimonas autotrophica.*

The bacterial community structure substantially changed in the BES units during the experiment. In the case of the two BES units (CO and IFO) relative abundance of these two dominant genera was altered, mainly due to an increase in the relative abundance of genus *Sulfurimonas*. In addition, in these BES units, genus *Arcobacter* abundance increased up to 5 times compared to the initial sample. In the BES unit with dispersant, the bacterial community structure was altered most strongly. Genus *Sulfurimonas* nearly disappeared and the community was dominated by the genus *Oleispira (O. antarctica)* and *Pseudomonas*.

6 DISCUSSION

Varying values of pollutant removal efficiency were observed across all three BES units at the end of the experiment. It could be assumed that a longer experimental duration would result in a higher degradation efficiency. Generally, oil biodegradation rates are determined by nonlinear models and thus exact predictions cannot be made based on our data.

The highest oil removal efficiency was observed in the BES unit (CO+D) across all the alkane lengths (C10 - C21, C21 - C40, C10 - C40). The increased level of oil degradation in this BES unit could be attributed to the efficiency of the dispersant Finasol 52. The addition of dispersant to the crude oil enhances the emulsification of oil breaking it down into more dispersed droplets and in this way the oil bioavailability to microbes increases. It has been shown in several studies that the application of dispersants enhances oil degradation in marine environments (Ferguson *et al*, 2017; Sun *et al*., 2019).

A comparison of the microbial community abundance and structure in our BES unit before and after the experiment depicts crucial findings worthy of mention. On day 0, the microbial community in the sediment sample was dominated by genus *Sulfurovum* with two species *Sulfurovum lithotrophicum* and *Sulfurovum aggregans,* and genus *Sulfurimonas* with dominating species *Sulfurimonas gotlandica* and *Sulfurimonas autotrophica.* We could observe at the end of our experiment that the somewhat equal distribution of *Sulfurovum* and *Sulfurimonas* was distorted in our CO and IFO units with *Sulfurimonas* occupying 45-60% dominance. Worthy of note also is the growth of *Acrobacter* with a 5 fold (up to 5%) rise in dominance from its initial abundance state on day 0. Moreso, the earlier observed dominance of *Sulfurimonas* changed drastically in our CO+D unit from 60% to 2%. *Sulfurovum* and *Sulfurimonas* are common microbial taxa near the hydrothermal deep-sea vents and in marine sediments playing important roles in biogeochemical processes involving sulfur reduction (Han & Perner, 2015). These genera are sulfur-reducing bacteria and their roles in the degradation of toluene have been investigated (Daghio *et al.*, 2016; Cruz Viggi al., 2015). *Sulfurimonas* have been also observed as an important group in oil biodegradation in seawater (Lofthus *et al*., 2020). So far, *Sulfurimonas* and *Sulforovum* have been found in bioelectrochemical systems treating wastewater (Li *et al*., 2021; Rossi *et al*., 2022; Zhang *et al*., 2021).

On the other hand, the specie *Pseudomonas* has been found in many studies about MFC applied for the biodegradation of oil hydrocarbons (Ferguson *et al*., 2017; Varjani & Upasani, 2016, Zang *et al*., 2005, Kaczorek & Olszanowski, 2010). Friman *et al*. (2012) alluded to the importance of *Pseudomonas putida* F1 in the bioelectrochemical system for toluene biodegradation supporting the fact that *Pseudomonas spp* which took over dominance in the CO+D unit at the end of the experiment is of great economical importance in the biodegradation of PHC.

The addition of dispersant led to the dominance of two genera- *Oleispira* and *Pseudomonas* in the BES unit. Such changes have been recorded also in other studies about the impact of dispersants on sediment bacterial community composition (Perez Calderon *et al*., 2018; Ferguson *et al*, 2017; Thomas *et al*., 2021).

The archaea community abundance in our BES CO+D sediment sample on day 0 before the start of the experiment was about 9% but surprisingly, at the end of the experiment run on day 34, the archaea community abundance had dropped in magnitude to 1% which represents a 90% decline in archaea community abundance. Most likely this happened due to either direct toxicity of the dispersant to archaea or due to the indirect impact of the dispersant as the dispersant makes oil more soluble in the sediment. The same kind of negative effect of dispersant on archaeal abundance has been observed in the case of Mediterranean deep-sea microbial communities (Liu *et al*., 2017). It has been suggested that biosurfactants (surfactants produced by bacteria) may be less harmful to the microbial community during oil biodegradation (Joe *et al*., 2019).

Conclusions

1. The bioelectrochemical system has good potential for the removal of oil compounds from heavily polluted marine sediments.
2. The oil biodegradation process in BES can be enhanced by the addition of dispersant.
3. Addition of dispersant Finasol 52 alters the bacterial community structure in BES and negatively affects the abundance of archaea.

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SUPPLEMENTARY

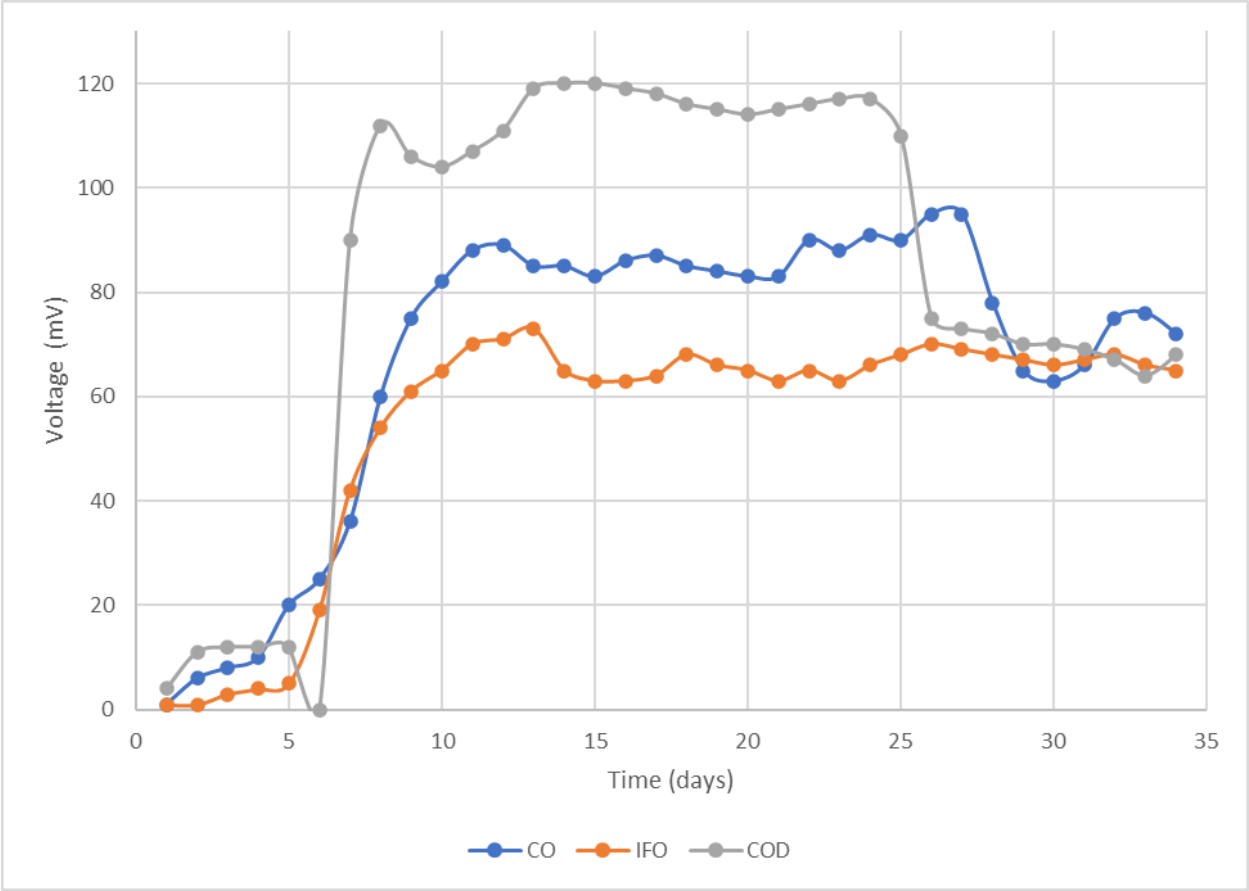

Figure S1. Voltage dynamics in BES units during the experiment.

Table S1. The abundance of bacteria and archaea in the sediment and BES unit samples according to quantitative PCR results. Shown is also the relative abundance of archaea in the prokaryotic communities. The sediment sample indicates the microbial abundance on Day 0 before spiking material with crude oil and IFO.

Sample	A16S (copies/g dw)	B16S (copies/g dw)	Total 16S rRNA genes (copies/g dw)	Proportion of archaea (%)
Sediment	$1.3x10^{10}$	$1.3x10^{9}$	$1.4x10^{10}$	9
CO	$1.7x10^{10}$	$2.2x10^{9}$	$2.0x10^{10}$	11
IFO	$2.1x10^{10}$	$2.4x10^{9}$	$2.4x10^{10}$	10
CO+D	$6.6x10^{9}$	$7.5x10^{7}$	$6.7x10^{9}$	1

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