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Bioremediation of Heavily Polluted Environment with Oil Spillage: Recent Advances

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BIOREMEDIATION OF HEAVILY POLLUTED ENVIRONMENT WITH OIL SPILLAGE: RECENT ADVANCES

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ABSTRACT

Oil spills provide unique challenges that must be managed since they represent several threats to the environment, the economy, and society. They have a negative influence on ecosystems and are difficult to clear up with traditional approaches. The effectiveness of many microbial species including bacteria, fungus, and algae to naturally breakdown is largely dependent on the oil's and the nutrients' bioavailability. Bioremediation using microorganisms to degrade heavily polluted environment contaminated with oil spillage, has emerged as a promising solution. In this review, recent advances in bioremediation for improved efficiency and effectiveness in heavily polluted environments with oil spill have been addressed. For instance, the use of genetically modified microorganisms, bioaugmentation, and biostimulation have shown significant improvements in oil degradation rates. One revolutionary method in this field of inquiry involves introducing novel materials into the polluted environment in order to biostimulate the therapy process. Another technique for increasing bioremediation is to use biosurfactants, which lower surface tension. Some polymeric compounds can be used to increase microbe immobilization, hence increasing the rate of breakdown. Microorganisms are manipulated to create novel bioaugmentation strategies that change enzymatic characteristics, modify metabolic pathways, increase substrate rate, and improve gene resistance to catabolic activities. Additionally, the application of nanotechnology, biosurfactants, bioelectrochemical system (BES) and polymeric materials has enhanced the bioavailability and biodegradation of oil pollutants. These advances have been successfully applied in various oil-spill affected sites, demonstrating the potential of bioremediation to restore ecosystems and promote environmental sustainability.

Keywords: Bioaugmentation, Biodegradation, Bioremediation, Biostimulation, Oil spillage.

1.0 INTRODUCTION

The wide-ranging and detrimental effects of environmental pollution on ecosystems, public health, mental health, and socioeconomic aspects necessitate fast and substantial restoration and rehabilitation actions (Mohammad *et al*., 2022). The need for a secure and healthful workplace has steadily increased throughout time. Both organic and inorganic pollutants are bad for the ecosystem and the health of people and animals. Heavy metals (HMs) are among the most notorious contaminants due to their defined character, non-biodegradability, and abundance (Hamza *et al*., 2023). Crude oil and refined gasoline spills have damaged natural ecosystems in many parts of the world, including Alaska, the Gulf of Mexico, the Galapagos Islands, France, the Niger Delta region of Nigeria, and many more (Godleads *et al*. 2015).

According to study, sea-based operations emit around 2 million tonnes of oil into aquatic habitats each year (Ajibade *et al*., 2021). Petroleum could remain in aquatic environments for long period following an oil spill (Jayasena and Perera, 2021). Petroleum hydrocarbon pollution has the potential to significantly harm the aforementioned ecosystems as well as human security because of its inherent toxicity and carcinogenicity, as well as its capacity for bioaccumulation, biomagnification, and resistance to biodegradation (Ajibade *et al*., 2021).

Natural gasses and aromatic, heterocyclic hydrocarbons combine to form petroleum, a complicated combination. The composition of crude oil, aside from being one of the most extensively utilized energy sources globally, is a complex and diverse blend of hydrocarbons, primarily alkanes, saturates, aromatics, resins, asphaltenes, naphthenes, and so on (Mohammad *et al*., 2022). The process of eliminating pollutants from a polluted environment through the use of microbial activity is known as bioremediation, and it is an important biotechnology technique. By increasing the bioavailability of the aforementioned contaminants for oleophilic microorganisms, biosurfactant—a powerful and environmentally benign compound with properties resembling those of surfactants generated by certain microorganisms—can improve the breaking of petroleum hydrocarbons (Mohammad *et al*., 2022).

2.0 NATURAL OR GENETICALLY MANIPULATED MICROORGANISMS

Local microorganisms are used in the natural bioremediation process to break down oil. Many species can naturally decompose oil in soil or water, including bacteria, fungus, and algae (Amber *et al*., 2021). The bioavailability of nutrients and oil plays a significant role in the efficiency of microbial decomposition. Increasing growth-limiting nutrients is a common method for improving bioremediation; however, this can result in a two-to-four weeks extension of cleaning time as well as an increase in the microbial lag time, which is the time before cells begin to multiply (Okoh *et al*., 2020).

Bioremediation may be hampered by chemical or mechanical remediation methods. While mechanical processes frequently entail localizing the oil, which can stymie the growth of many germs due to a lack of resources such as sunlight and oxygen, chemical treatments may prevent bacteria from degrading the oil by making it more lethal (Hoang *et al*., 2018). Bio-augmentation, thanks to the advent of genetically modified microbial strains, has emerged as a viable solution to the problem of slow and inefficient waste and pollution degradation.

Bioremediation is the only method for cleaning up contaminated areas that has been shown to be safe, affordable, and long-lasting (Dai *et al*., 2020; Singh *et al*., 2021). This is why it takes a lot of work to choose the right microbial strain and then design it. In environmental biotechnology, genetically modified microorganisms (GEMs) are being utilized to get rid of pesticides, xenobiotics, and toxic pollutants (Pant *et al*., 2021). Understanding natural microbial communities is necessary before creating a synthetic one. Finding the species performing bioremediation in a natural setting might be difficult (Nwankwegu *et al*., 2022).

It is possible to create an artificial microbiome for bioremediation that contains functionally specialized species by using a synthetic biome, which is created by cultivating two microbial species under specific conditions based on their interaction and serves as a model system for functional, structural, and ecological aspects (Anjum *et al*., 2022). There are two possible outcomes from social interactions (such mutualism, cooperation, and competition, for instance) between two microbial populations: +/+ and/+ or +/. It is believed that cooperation is crucial to the makeup and functioning of communities (Saleh *et al*., 2022).

Any creature can be changed into the desired shape using recombinant DNA technology (Haritash, 2020; Landa-Acuฤna *et al*., 2020). The desired gene can then be inserted into the genome of the vector (such as a phage, plasmid, or virus) to produce an appropriate host gene in a different creature (Patel *et al*., 2022). This approach facilitates the bioremediation process. Among the many instruments needed are restriction enzymes, DNA ligase, reverse transcriptase, alkaline phosphatase, T4 polynucleotide kinase and other DNA ligases, and host (Pal *et al*., 2020).

3.0 ROLE OF BIOSTIMULATION IN BIOREMEDIATION OF OIL SPILLAGE

Many factors, including nutrients, pH, temperature, moisture, oxygen, soil features, and contamination, may restrict hydrocarbon biodegradation in soil (Godleads *et al*., 2015). Biostimulation is the process of altering the surroundings to support pre-existing microorganisms with the ability to carry out bioremediation. Biostimulation is a form of bioaugmentation in which nutrients are added to boost the activity of native degrading bacteria, is a superior tactic to deal with some of these problems (Varjani *et al*., 2019). This accelerates the rate of microorganism degradation by increasing the concentration of microbial cells. Furthermore, extracting potential biodegraders from their native microbial populations and cultivating them in a laboratory environment can enhance the biodegradation rate (Bradford *et al*., 2018).

According to recent research by Ali *et al*. (2020), a saturated sample of desert soil with 17.3%, w/w crude oil might have 53–66% of the oil removed by certain identified bacterial species. A variety of bacteria that break down hydrocarbons and microorganisms that metabolize nitrogen were encouraged to proliferate when nutrients like $(NH_4)_2SO_4$, 0.43 g/kg soil and KH_2PO_4, 0.067 g/kg soil were added to crude oil-contaminated soil, according to Cai *et al*. (2020). The application of bioaugmentation, biostimulation, and natural attenuation processes in the bioremediation of soil polluted by crude oil was studied by Yaman *et al*. (2020). Biostimulants such potassium phosphate (KH2PO4) and ammonium chloride (NH4Cl) were employed to achieve a C:N:P ratio of 100:5:1. The results demonstrated the crucial importance of these two strategies for the bioremediation of soil polluted by crude oil; mixed bioaugmentation accounted for 74% of the total petroleum hydrocarbon, while biostimulation accounted for 66%.

Biostimulation has various functions in the bioremediation of oil spills, such as:

i. Biostimulation promotes the growth of oil-degrading microorganisms, increasing their metabolic activity and accelerating the breakdown of hydrocarbons present in the spilled oil.

ii. Biostimulation has been shown to significantly decrease the leaching of contaminants, including non-aqueous phase liquid (NAPL), into water, thereby preventing groundwater contamination.

iii. By enhancing the biodegradation of oil in the mobile phase, biostimulation can reduce or even prevent the migration of oil in recently contaminated areas, contributing to the overall cleanup of the site.

iv. Biostimulation is considered a potentially safe in situ treatment for oil spillage remediation, as it utilizes natural processes and microbial activity to degrade contaminants without causing harmful side effects on the environment.

4.0 ROLE OF BIOAUGMENTATION IN BIOREMEDIATION OF OIL SPILLAGE

Bioaugmentation, the process of introducing specially created microbes to contaminated areas so they might consume harmful pollutants. Bioaugmentation increases the breakdown of complex pollutants by boosting the type and quantity of pollutant-degrading microorganisms

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(Omokhagbor Adams *et al*., 2020). Bioaugmentation is an inexpensive, rapid, and effective bioremediation method (Mahmoud, 2021). Allochthonous bacteria are added to contaminated areas to supplement the local microorganisms. In some cases, it can also mean taking microorganisms out of the contaminated area and changing their genetic composition before reinstalling them in order to facilitate rehabilitation.

Because resident microorganisms at contaminated areas may not naturally be able to degrade the contaminant present at a place, their ability to do so is enhanced by genetic manipulation. In certain instances, non-resident microorganisms are introduced to contaminated sites in order to facilitate the breakdown of contaminants. The capacity of these novel strains to compete with the local bacteria and adapt to the new environment are two criteria that will determine their efficacy (Babalola *et al*., 2019). When applied to a polluted site, *Burkholderia* sp. FDS-1 has been shown to reduce the harmful effect nitrophenolic molecule at a mildly acidic pH and a temperature of roughly 30° C found in pesticide-polluted soil (Ojuederie).

Chaudhary *et al*. (2021) investigated the potential use of bioaugmentation to remediate soil polluted by diesel fuel in the distant past by adding nutrients and zero-valent iron nanoparticles. Protobacteria made up 64.69% of the bacterial consortium. The results showed that 99% of the petroleum hydrocarbons in the soil had broken down after 60 days (Chaudhary *et al*., 2021). In order to do off-site field bioremediation, Varjani and Upasani (2016) investigated *Pseudomonas aeruginosa* NCIM 5514 species that was extracted from soil contaminated with petroleum. During the process of ex-situ bioremediation, the species showed an approximately 60.63% hydrocarbon degradation capacity. Based on these findings, *P. aeruginosa* may be used to mineralize PHC contaminants both ex-situ and in-situ (Varjani and Upasani, 2016). The bioaugmentation techniques is described in Fig. 1 below.

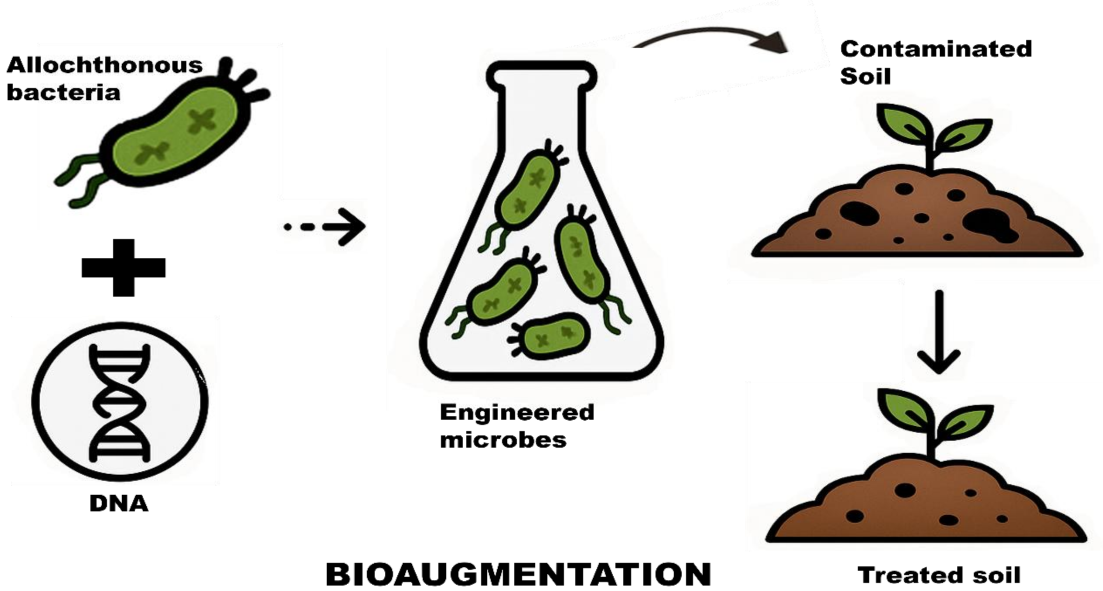

Fig. 1: Bioaugmentation

5.0 ROLE OF BIOSURFACTANT IN BIOREMEDIATION OF OIL SPILLAGE

In hydrocarbon bioremediation, surfactants are used to liberate the hydrocarbons for microbial breakdown. Thus, bulk transfer of the hydrocarbons to the aqueous phase is an essential step in guaranteeing their bioavailability (Adrion *et al*., 2016). Among many strategies, surfactants are considered the most promising for resolving bioavailability-related problems. Haftka *et al*. (2015) claim that adding surfactants may increase the hydrocarbons' mobility and bioavailability and quicken their rate of biodegradation. Cheng (2016) claims that the petroleum industry has been the main user of surfactants due to their potential to increase the solubility of gasoline and its byproducts. Based on structural differences, the different groups of surfactants are divided according to the type of microorganisms that produced them (Cheng *et al*., 2016).

Biosurfactants are bioactive surface molecules generated by microorganisms with a wide range of applications due to their unique, adaptable properties, minimal toxicity, and biological acceptance (Shivlata & Satyanarayana, 2015). They work on the synthesis of organic compounds, petrochemicals, and petro-derivatives. Biosurfactant-producing bacteria have the potential to successfully bioremediate waste water effluents because of their special ability to exploit organic and hydrocarbon waste as source materials (Tiquia-Arashiro and Rodrigues, 2016).

Higher surface activity biosurfactants are more resistant to a range of environmental conditions, including temperature, salt concentration, ionic strength, biodegradability, basicity or acidity of an aqueous solution, and antibacterial activity, according to Rodriques *et al*. (2015). Bacteria classified as extremophilic thrive in harsh environments. Since they produce significant substances that offer them unique abilities, they have attracted a lot of attention lately (Sarma and Prasad, 2015). To encourage the biosorption of polyaromatic cyclic molecules, screening for biodegradable surfactants from harsh marine environments is crucial because chemically produced surfactants present significant environmental concerns (Neitsch *et al*., 2016). Tiquia-Arashiro and Rodrigues (2016) describe biosurfactants as amphiphilic compounds having a hydrophobic moiety made up of hydroxylated, saturated, or unsaturated fatty acids and a hydrophilic polar moiety made up of proteins, polysaccharides, peptides, oligo or monosaccharides. The primary criteria used to categorize biosurfactants are their chemical composition and place of origin (Shivlata and Satyanarayana, 2015).

The hydrophilic-lipophilic balance, an essential property of biosurfactants, defines the hydrophobic and hydrophilic components of surface active substances. Because biosurfactants are amphiphilic, they can increase the surface area of hydrophobic substances while also changing the surface properties of microorganism cells. Because of their surface activity, surfactants are good emulsifiers, foamers, and dispersants (Sarma & Prasad, 2015). Biosurfactants aid in the selective breakdown of substrates and demonstrate functional activity in harsh circumstances like high pH, high temperatures, and high salt concentrations—all of which are associated with industrial waste and commodities.

According to Adrion *et al*. (2016), surfactants have a variety of properties, including coating, foaming, emulsification, dispersion, and wetting. By creating metal compounds and removing heavy metals from the surface, biosurfactants can increase metal ion concentrations and bioavailability in heavy metal-polluted soils (Neitsch *et al*., 2016). Cheng *et al*. (2016) claim that surfactants, such as pesticides sprayed on water and soil, increase the hydrophobic particles' surface area and solubility. The development of microbial surfactants and their widespread application in the breakdown of pesticides and herbicides has attracted increasing attention in recent years.

6.0 ROLE OF POLYMERIC MATERIALS IN THE IMMOBILIZATION OF MICROORGANISMS AND BIODEGRADATION

Organic acids including citric, malate, and acetic acids improve metal solubility, mobility, and absorption (Saha *et al*., 2021). Polymeric compounds including polyesters, polysaccharides, and polyphosphates enhance metal phytostabilization and hence assist bioremediation (by mobility). Similarly, hydrophobic substrates are assisted by biosurfactants like gramicidin, viscosin, polymixin, and glycoprotein in becoming more soluble, mobile, and bioavailable.

Another effective approach for increasing biodegradation is to immobilize microorganisms used in bioremediation. The most common way of immobilization, known as biofilm growth, encases and traps bacteria in polymeric gels. To extract petroleum oil from artificial saltwater, Ayilara and Bablola (2023) used polyurethane foam to immobilize a coculture of *Gordonia* sp.and *Pseudomonas monteilii*. Polyurethane foam was chosen as the carrier material because it is widely available, inexpensive, and has great buoyancy and oleophilic properties.

In a recent study, several temperatures have been investigated. The best oil removal happened after 7 days of operation at 30°C with immobilized mixed biofilm on polyurethane foam. Biological activity and sorption on the biofilm/carrier system were coupled to extract the oil. Alternatively, the immobilized cell might be retained. *P. monteilii* was maintained at 4 °C, which resulted in an increase in oil bioremoval at low temperatures even though the bacteria's viability in the biofilm reduced. They concluded that bacteria adapt to low temperatures during storage and become more metabolically active (Ayilara and Bablola, 2023).

7.0 MODES OF ACTION/MECHANISMS OF MICROBIAL BIOREMEDIATION

In many different methods, microbes may eliminate contaminants from their environment. These processes are separated into two categories by Verma and Kuila (2019): immobility and mobilization. Enzymatic reduction, bioleaching, bioaugmentation, biostimulation, and enzymatic oxidation are the steps in the mobilization process. Numerous mechanisms, including bioaccumulation, biosorption, complexation, and precipitation (solidification), can lead to immobilization. Microbes transform contaminants into different end products like water and carbon dioxide, as well as metabolic intermediates. On the other hand, immobilization is the process of modifying a chemical's structure such that it can no longer exist in its native environment. Think about how organic nitrogen is created from nitrate nitrogen (Pratush *et al*., 2018).

Pratush *et al*. (2018) state that immobilization can be carried either ex-situ or in-situ. Ex-situ treatment involves taking contaminated soils out of their original location and relocating them to another location where the metal ions causing the pollution are rendered immobile using a microbiological technique. Contrarily, the in-situ method addresses pollution at its source (Cao *et al*., 2020). Microbes like *B. cereus* and *E. asburiae* have been shown to partly immobilize environmentally toxic heavy metals (Fashola *et al*., 2020). In order to protect themselves from dangerous compounds, microorganisms during microbial bioremediation produce solvent efflux pumps which are hydrophobic that coat the cell's outer membrane (Verma and Kuila, 2019). The mechanisms of microbial bioremediation are highlighted in Fig. 2 below.

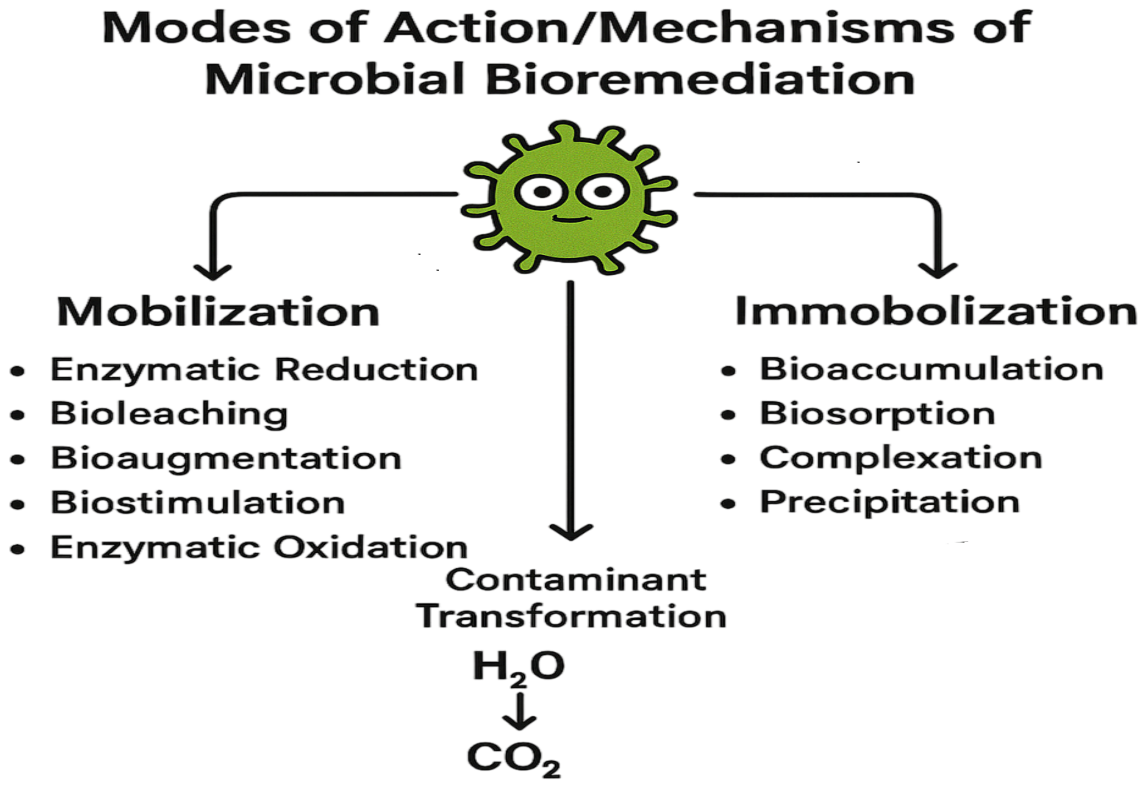

Fig. 2: Modes of Actions of Microbial Bioremediation

8.0 MICROBIAL ENZYMES IN BIOREMEDIATION

Variety of enzymes from microbes have been found to be useful in removing pollutants from the environment (Bhatt *et al*., 2021). Enzymes in bioremediation utilize methods such oxidation, reduction, and elimination (Bhandari *et al*., 2021).

8.1 Enzymatic Oxidation

Enzymatic oxidation lowers harmful compounds from a higher to a lower oxidation state, which causes heavy metals to lose an electron and part of their effectiveness. The enzyme oxidoreductase, which is produced by the bacteria involved, is required for this process. Chaudhary *et al*. (2023) state that this technique is particularly effective for getting rid of dyes, phenols, and other contaminants that are difficult for microorganisms to break down. By producing radicals, oxidative enzymes may break down molecules into various portions and create compounds with a high molecular weight (Bhatt *et al*., 2021). The oxidation of aromatic amines is catalyzed enzyme oxidoreductase laccase. Phenols and polyphenols are two further examples of compounds that convert molecular oxygen into water. Laccase formation has been seen in *Leptosphaerulina* sp. and *Pycnoporus* sp., which are thought to digest heavy metals (Copete-Pertuz *et al*., 2018; Tian *et al*., 2020). Enzymatic oxidation process of microdial bioremediation is described as seen as Fig. 3 below.

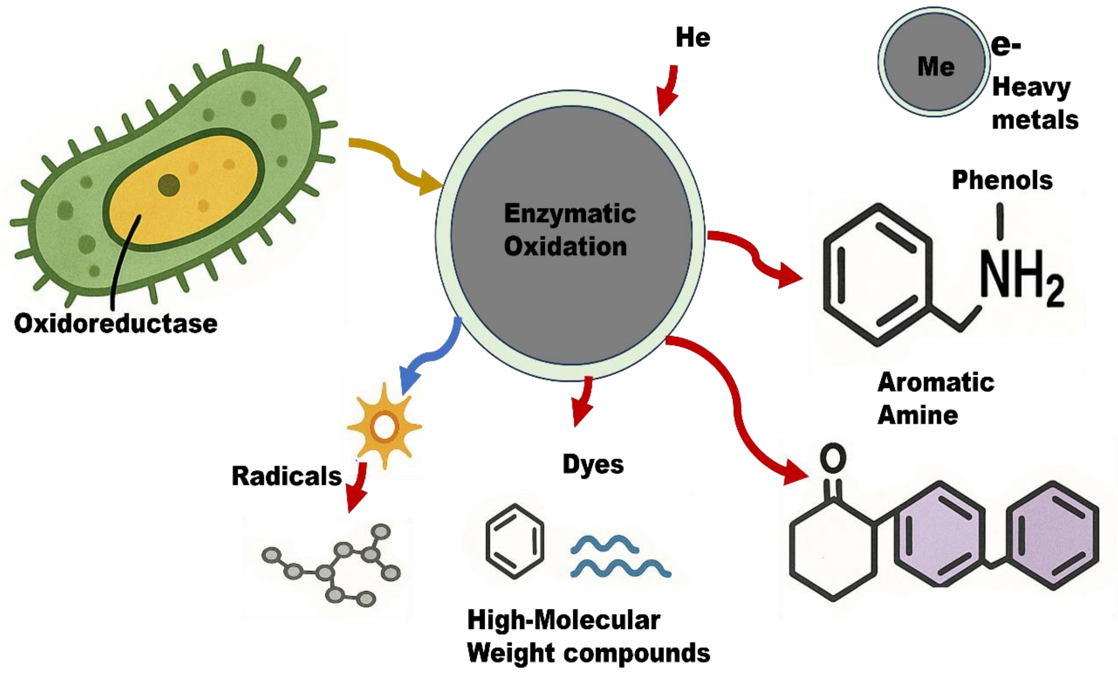

Fig. 3: Enzymatic Oxidation Process of Bioremediation

8.2 Enzymatic reduction

During the process of enzymatic reduction, pollutants are reduced to an oxidized state and become insoluble. The process is applicable to facultative as well as necessary anaerobes and is useful for the clean-up of pollutants such as polychlorinated dibenzo-p-dioxins and dibenzofurans (Zacharia, 2019). Analogously, azoreductase transforms azo compounds into azo links, whereas chrome reductase reduces hexavalent chromium to trivalent chromium (Saxena *et al*., 2020). To find out which of the numerous creatures in our environment can bioremediate pollutants, more study is required.

9.0 ELECTROCHEMICAL METHODS AND BIOLOGICAL ROUTES IN BIOREMEDIATION OF OIL SPILLAGE

Bioelectrochemical systems show great potential as a technology capable of harnessing electrons to drive reduction-oxidation reactions on electrodes (Yang *et al*., 2019). These systems have the capability to convert the chemical bond energy directly into electrical energy of organic compounds, bypassing the need for additional intermediate stages (Kumar *et al*., 2017). Electroactive bacteria in a BES break down the substrate to create protons and electrons (Leiva *et al*., 2018). An external connection facilitates the flow of electrons from the anode to the cathode. Protons and electrons then undergo chemical reactions on the surface of the cathode. BESs, which have the ability to oxidize a range of refractory organic molecules, depend heavily on microbial anodes (Rossi *et al*., 2018).

9.1 Role of Bioelectrochemical Systems (BES) in Bioremediation of Oil Spillage

Bioelectrochemical systems operate under more benign and straightforward circumstances than conventional electrochemical systems. There are many different types of organic compounds suitable for use as substrates (Beretta *et al*., 2019). They can be utilized as catalysts in the electrochemical process more frequently, which lowers costs, because precious metals are rarely required in BESs (Leon-Fernandez *et al*., 2019). Bacteria are fundamental to microbial remediation and are crucial to BESs. Different BESs and contaminants have varying capacities for the EET that bacteria can conduct. Furthermore, the various BES varieties may remediate pollutants in different ways. This section provides a summary of the EET and related types of BES (He *et al*., 2022).

The utilization of biological metabolic processes to speedup the breakdown of petroleum contaminants into CO_2 and water is known as currently "bioremediation technology", which is thought to be an economical and environmentally beneficial way to treat petroleum oil spills (He *et al*., 2022). This process can take place in both anaerobic and aerobic environments (Li *et al*., 2021). The optimal electron acceptor for bacteria is O2, which also establishes the pollutant degradation pathway (Zhou *et al*., 2018). Aerobic bacteria use plentiful O2 as electron acceptors during aerobic breakdown. However, as oxygen is quickly exhausted, anaerobic bacteria finally take over. Anaerobic degradation results in the slow and selective breakdown of petroleum hydrocarbons because microorganisms have limited access to terminal electron acceptors (NO^{3-}, SO_4^{2-}, Fe^{3+} and Mn^{4+}). (He *et al*., 2022). According to He *et al*. (2022), the sand column containing crude oil was subjected to a carbon dioxide production rate measurement. The findings indicated that the rate of carbon dioxide generation was significantly higher in aerobic settings compared to anaerobic conditions. Nevertheless, anaerobic degradation is still a significant method of the natural breakdown of petroleum hydrocarbons in anoxic settings such deep soil, groundwater, or sediment. Thus, microbial activity in their natural habitat can be increased through biological stimulation through the introduction of electron receptors (Li *et al*., 2021).

This restriction has recently been overcome by bioelectrochemical systems (BESs) based on microbial technology, accepting electrons produced during substrate utilization and encouraging the metabolic transformation of pollutants in close proximity to the anode (Roy, 2023). BESs have a great deal of potential for cleaning up soil, sediment and groundwater contaminated by petroleum (Li *et al*., 2021). According to Li *et al*. (2021), in 2009, the viability of using BESs for groundwater hydrocarbon bioremediation was initially confirmed. According to the results, after 21 days, the diesel in BES had degraded at a rate of 82%, which was significantly higher than the control group's 31% diesel degradation rate at an initial concentration of 300 mg/L. Sewage remediation has been a successful use of this technology in recent years. The results demonstrated that, after 21 days, the diesel in BES had degraded at a rate of 82%, which was much higher than that of the control group (31%), which had started with a baseline concentration of 300 mg/L. Recent, this method has been successfully used to clean up sewage (Li *et al*., 2021), sediment/sludge (Li *et al*., 2023) and contaminated soil (Wang *et al*., 2021). Therefore, BESs offer a potential solution for environmental petroleum hydrocarbon remediation (Li *et al*., 2021).

9.2 Microbial Fuel Cells for Conversion of Chemical Energy into Electricity Concurrent with Contaminant Degradation.

Microbial fuel cells (MFCs) are BESs that do not require an external power source (Jung *et al*., 2020). *Saccharomyces cerevisiae* was found by Potter in 1910 to be capable of transferring intracellular electrons to external solid electron receptors, such as electrodes or minerals. Potter discovered that microorganisms could produce electricity a year later (Li *et al*., 2020). A growing number of researchers started to focus their efforts on the field of BESs coinciding with advancements in fuel cell technology during the early 1990s, (Yap and McLellan, 2023).

A microbial fuel cell's anodic and cathodic chambers are separated using proton exchange membranes (PEMs) (Gul and Ahmad, 2019). MFCs operate on the biocatalytic potential of electroactive bacteria (EAB), which decompose organic materials and generate bioelectricity, as seen in Fig. 1 (Kumar *et al*., 2019).

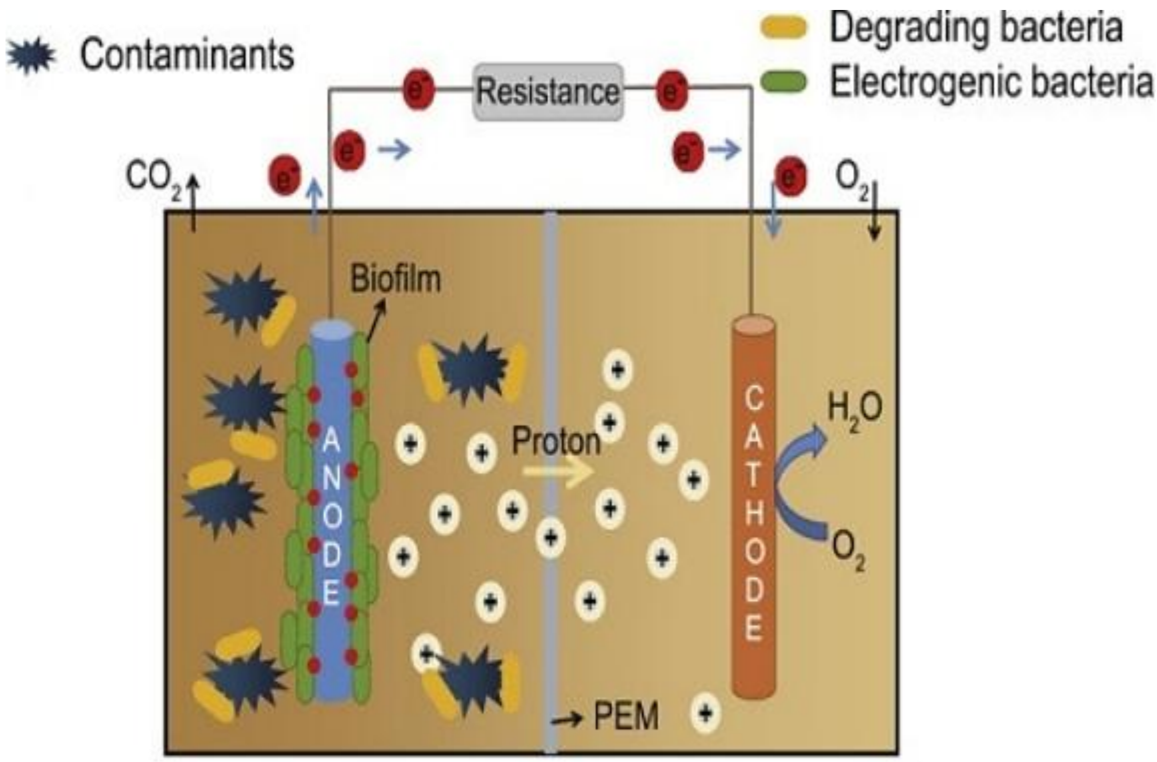

Fig. 4: Schematic diagram of MFCs (Kumar *et al*., 2019).

Bacteria oxidize the substrate on the surface of the anode to produce protons and electrons with EET capacity. The external circuit is then used to transmit the electrons to the cathode. The reaction of electrons, protons, and oxidants ultimately forms stable reduction products on the surface of the cathode (Li *et al*., 2021). The efficacy of the power of MFC generation and pollution removal is significantly impacted by the performance of the membranes (Koók *et al*., 2019). Membranes serve to prevent the mixing of solutions between the two chambers (Bakonyi *et al*., 2020). The infiltration of anodic solutions to the cathode side can lead to significant biofouling on the cathode surface, resulting in the deterioration of MFC performance (Li *et al*., 2021). The absence of membranes allows oxygen from the cathode chamber to move to the anaerobic anode chamber, thus inhibiting the anaerobic fermentation process (Fatehbasharzad *et al*., 2022). Nafion membranes are widely preferred as proton exchange membranes (PEMs) in MFCs due to their superior proton conductivity and adequate ion exchange capacity (Sharma *et al*., 2021). Nafion membranes have not been widely used for scaling up MFC applications due to a number of issues, including oxygen leakage, substrate loss and crossover, and the potential for biofouling (Li *et al*., 2021). To enhance MFC performance, researchers have developed a variety of membrane types. Anion exchange membranes (AEMs), cation exchange membranes (CEMs), and bipolar membranes are the three types of membranes. Cation transfer in CEM is achieved by connecting negatively charged functional groups to a backbone. One type of CEM membrane is sulfonated polyetheretherketone, or SPEEK (Shabani *et al*., 2019), Poly (vinylidenefluoride) (PVDF) membranes, PES-SPES, CMI-7000, and polyethersulfone resin (PES) membranes (Li *et al*., 2021). Positively charged functional groups are added to the backbone of AEM to aid in the transport of anions (Treichel *et al*., 2022). Despite having benefits including higher buffering, lower resistance, and a pH restriction decline across the membrane, AEMs are less popular in MFCs than CEMs (Fatehbasharzad *et al*., 2022). After then, ion exchange is limited between the anode and the cathode as OH^- travels through AEM to the anode and H^+ travels via CEM to the cathode. The pH variations that various membranes will produce in the anode and cathode chambers have an effect on MFC performance. As such, different membranes should be selected for different types of pollutants (Wang *et al*., 2019).

Serious soil pollution has led to the usage of MFCs in soil remediation in recent years. New remediation techniques called "soil MFCs" generate energy while using BESs to break down and remove pollutants from the soil, including pesticides, heavy metals, petroleum hydrocarbons, and antibiotics (Li *et al*., 2021). Soil MFCs do not require the high energy consumption of standard physicochemical approaches, nor the addition of catalysts, chemical oxidants or solvents (Wang *et al*., 2019). Figure 2 illustrates one of the standard air cathodes that MFC utilizes to remediate oil-polluted soil. An external circuit connects the air cathode with the anode that is placed in the soil environment to create a closed circuit. To release protons and electrons during the decomposition of soil contaminants, electrogenesis bacteria catalyze the process of pollution remediation (Abd-Elrahman *et al*., 2022).

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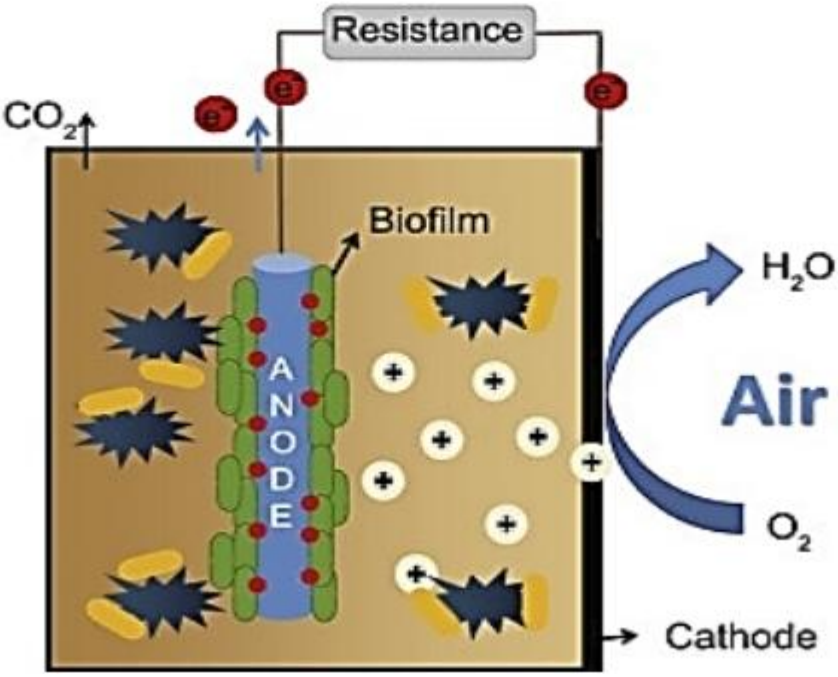

Fig. 5. Schematic diagram of MFCs (standard air cathodes) (Li *et al*., 2021).

Currently, there are various types of soil MFCs available, such as plant MFCs, graphite rod air-cathode soil MFCs, dual-chambered MFCs, U-tube MFCs, sediment MFCs, column-type MFCs, three-chamber MFCs, and insertion-type air-cathode soil MFCs (Li *et al*., 2021).

10.0 CONCLUSION AND FUTURE RESEARCH ON THE OIL SPILL BIOREMEDIATION

The utilization of BESs offers a sustainable, versatile and environmentally friendly approach for remediating soil contaminated with petroleum. Furthermore, this technology ensures that the future usability of the land remains unaffected post-remediation. Redox reactions occurring around the electrode efficiently remove pollutants. Past successful applications of microbial electrochemical techniques serve as valuable references across various aspects, while research and literature on BES applications in other mediums should be consulted to optimize BES utilization in soil remediation. Microbial electrolysis cells (MECs) are more complicated to produce and require extra energy sources for site cleanup than microbial fuel cells (MFCs), which generally have more potential for this purpose. As a result, MFCs provide clear benefits over MECs in terms of operation and maintenance.

Conclusively, the development of any technology requires ongoing optimization and improvement in order to go from research to application.

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